THE CHEMISTRY OF SEED OILS: WITH PARTICULAR REGERENCE TO OILS CONTAINING HYDROXY AND/OR EPOXY ACIDS

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A Thesis Submitted for the Degree of PhD
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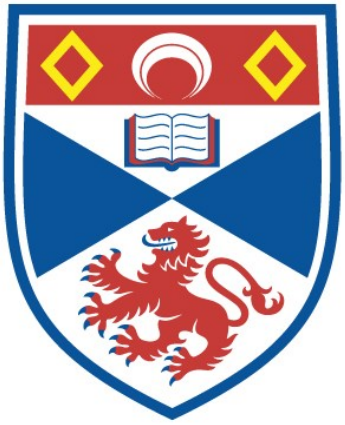

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THE CHEMISTRY OF SEED OILS

(With Particular Reference to Oils Containing Hydroxy and/or Epoxy Acids)

being a Thesis

presented by

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DECLARATION

I hereby declare that the following Thesis is based on the results of experiments carried out by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The Research was carried out in the Chemical Research Laboratories of the University of Glasgow (October 1953 to September 1954) and of St. Salvator's College, University of St. Andrews (October 1954 to July 1956) under the direction of Dr. F. D. Gunstone.

CERTIFICATE

I hereby certify that Mr. Khurshed Erachsha Bharucha has spent nine terms at Research Work under my direction*, and that he has fulfilled the conditions of Ordinance No. 16 (St. Andrews and that he is qualified to submit the accompanying Thesis in application for the Degree of Doctor of Philosophy.

Research Supervisor.

* The candidate has worked for three terms in the University of Glasgow (October 1953 to September 1954) but has permission of the Senate to submit his Thesis after six terms at the University of St. Andrews.

UNIVERSITY CAREER

I entered Wilson College, Bombay, India, in 1945 and graduated B.Sc.(Hons.) with Second Class in Chemistry in 1949. I then joined the Department of Chemical Technology of the University of Bombay and graduated B.Sc.(Tech.) in 1951 with First Class in Technology of Oils, Fats and Waxes. In 1954, I wa awarded the degree of M.Sc.(Tech.) by the same University on the basis of a Thesis entitled "Recovery of Nickel from Spent Nickel Catalysts", work for which was carried out at Bombay from July 1952 to July 1953.

The research described in this Thesis was carried out in th Chemical Research Laboratories of the University of Glasgow (October 1953 to September 1954) and of St. Salvator's College, University of St. Andrews (October 1954 to July 1956) under the direction of Dr. F. D. Gunstone. I was appointed in 1955 to a University Scholarship.

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The Chemistry of Seed Oils (With Particular Reference to Oils Containing Epoxy and/or Hydroxy Acids).

Part I. Study of Fatty Oils Containing Epoxy and/or Hydroxy Aci

An analytical procedure has been devised for the analysis of oils containing epoxy and/or hydroxy acids, and this has bee applied to the examination of Vernonia anthelmintica, Cephalocroton cordofanus and Strophanthus hispidus seed oils, and to Ergot oil.

The unusual acid present in C. cordofanus seed oil has bee shown to be cis-12:13-epoxyoctadec-cis-9-enoic acid, identical with the acid in V. anthelmintica seed oil, and not ricinoleic acid as previously reported.

The hydroxy acid present in Ergot oil has been shown to be identical with natural ricinoleic acid.

Attempts have been made to improve the method of determination of α-dihydroxy acids in oils and fats by periodat oxidation.

Part II. The Preparation of Eight 9:10:12:13-Tetrahydroxystear Acids.

The availability of cis-12:13-epoxyoctadec-cis-9-enoic ac has opened a new way for the preparation of 9:10:12:13-tetrahydroxystearic acids, hitherto prepared from linoleic aci

The epoxyoleic acid has been converted to threo- and erythro-12:13-dihydroxyoctadec-cis-9-enoic acids and these on oxidatio with either dilute alkaline potassium permanganate or with performic acid affords 9:10:12:13-tetrahydroxystearic acids. Eight tetrahydroxystearic acids have thus been prepared and these, like the starting materials are optically active.

Publications:

(i) Vegetable Oils. IV.- A New Method of Determining the Component Acids of Oils Containing Epoxy and/or Hydroxy Acids.
K. E. Bharucha and F. D. Gunstone, J. Sci. Food Agric., 1955, 6, 373.

(ii) Fatty Acids. Part IV.- The Preparation of Eight 9:10:12:13-Tetrahydroxystearic Acids.
K. E. Bharucha and F. D. Gunstone, J. Chem. Soc., 1956, 1611.

(iii) Vegetable Oils. V.- The Component Acids of Cephalocroto cordofanus Seed Oil.
K. E. Bharucha and F. D. Gunstone, J. Sci. Food Agric., accepted for publication.

(iv) Vegetable Oils. VI.- Ergot oil.
To be published.

Part I. Seed Oils Containing Epoxy and/or Hydroxy Acids.

Part IA. Introduction

(Epoxy and Hydroxy Acids Occurring in Nature)

Epoxy Acids

Vernonia anthelmintica seed oil was shown by Gunstone[1] (1954) to contain cis-12:13-epoxyoctadec-9-enoic acid (74%) and not an isomer of ricinoleic acid (12-hydroxyoctadec-9-enoi acid) as previously reported[2]. Thus for the first time an epoxy fatty acid was proved to occur naturally in a fat. The findings of Gunstone were later confirmed by Raman[3]. During the present investigation Cephalocroton cordofanus seed oil was found to contain the same epoxyoleic acid (66%) and not ricinoleic acid as reported by Henry and Grindley[4]. Yet another source of the same epoxy acid is Okra seed oil[5] which however, contains only minor amounts (3-4%) of this epoxyoleic acid. At present no other fat is known to contain naturally occurring epoxy acids, but in view of the fact that the same epoxyoleic acid has been isolated from three widely different species it is possible that epoxy acids are more widely distributed than is known at present.

Epoxy acids can be easily prepared by reaction of olefini acids with acids such as peracetic, perbenzoic, performic, etc To-day there is considerable interest in epoxidation and the reaction is utilised in the commercial production of a number of useful organic chemicals from olefinic raw materials. These are used as plasticizers, stabilizers, varnishes, resins, paints, lubricants, etc.. In a recent method[6] hydrogen peroxid in conjuction with a cation exchange resin is used for the

epoxidation of vegetable and animal fats and their derivatives. As V. anthelmintica and C. cordofanus seed oils contain high proportion of the epoxy acid they may be of commercial value.

Hydroxy Acids

With few exceptions long-chain hydroxy acids do not occur in abundant quantities among the component acids of fats. They occur, however, frequently and sometimes in significant amounts in wool wax, in insect waxes, in bacterial waxes, in waxes of coniferous plants, in brain lipids, in cork, etc.. Many of the naturally occurring hydroxy acids are optically active as the carbon atom to which the hydroxyl group is attached is asymmetr A large number of hydroxy acids have been synthesised but only those occurring naturally are here described.

Hydroxy Acids Occurring in Fats:

Ricinoleic acid (12-hydroxyoctadec-9-enoic acid). This is the most important of the hydroxy acids and though occurring mainly in fats of the Ricinus species is also present in fats of other species. The mixed acids of castor oil contains 90% or more of this acid from which it was, apparently, first isolated by Saalmuller[7]. The present structure was first assigned by Goldsobel[8] and confirmed by Walden[9] in the same year and by several workers[10] since then. Dehydration of ricinoleic acid results in the formation of a mixture of conjugated and non-conjugated octadecadienoic acids and dehydrated castor oil is hence used as a substitute for tung oil in drying oils. Ivory wood oil[11] (Agonandra brasiliensis) is reported to contain ricinoleic acid (47%) and this was also considered to be the major acid of Cephalocroton cordofanus

seed oil[4]. In the present work, however, C. cordofanus seed oil was proved to contain cis-12:13-epoxyoctadec-9-enoic acid as its major component; ricinoleic acid is considered to be absent. Several workers[12] have reported the occurrence of ricinoleic acid in ergot oil though on insufficient evidence. A sample of ergot oil examined during the present investigation was found to contain a hydroxy acid (34%) and this was proved to be ricinoleic acid. Ricinoleic acid (9.8%) is stated to be present in Argemone oil[13] and according to Margaillan[14] the oil of Wrightia annamensis contains as its chief component acid a hydroxymonoethenoid acid, probably ricinoleic acid.

9-Hydroxyoctadec-12-enoic acid has been shown by Gunstone[15] to occur in several seed fats of the Strophanthus species.

A hydroxy acid of unknown structure has been reported to occur in grape seed oil[16] but this claim has been refuted by others[16a]. An isomer of ricinoleic acid is believed to be present in quince seed oil[17].

Kamlolenic acid (18-hydroxyoctadeca-9:11:13-trienoic acid From the seed fat of Mallotus philippinensis an unsaturated solid acid, m.p. 77-78° was isolated by Gupta, Sharma and Aggarwal[18], and this when irradiated with ultra-violet light in presence of iodine gave an isomeric acid, m.p. 88-89°; the two acids were termed α- and β- kamlolenic acids. Based on reduction, oxidation and other studies, the acid was considere

to be either, 18-hydroxyoctadeca-9:13:15-trienoic acid or 18-hydroxyoctadeca-9:12:14-trienoic acid but Puntambekar[19] considered the acid to be 3-keto-octadeca-5:7-dienoic acid or 8-keto-octadeca-9:11:13-trienoic acid. Calderwood and Gunstone[2] proved kamlolenic acid to be 18-hydroxyoctadeca-9:11:13-trienoi acid. Later on Ahlers and Gunstone[21] from infra-red measurement showed α-kamlolenic acid to have one cis- and two trans-ethylenic bonds (probably cis-9, trans-11, trans-13) whereas the β- acid was the wholly trans- isomer. These findings have been confirmed by Mikush[22] and by Crombie and Tayler[23]. Recentl Toyama and Takai[24] isolated kamlolenic acid from Mallotus japonicus seed oil.

Isanolic acid (8-hydroxyoctadec-17-en-9:11-diynoic acid). This acid forms about 44% of the mixed acids of boleka or isan oil, the seed fat of Onguekoa Gore. Riley[25] has established th position of the hydroxyl group at carbon 8 and from the similarity of its ultra-violet spectra to that of isanic acid, Kaufmann et.al.[26] consider it to be 8-hydroxyoctadec-17-en-9:11-diynoic acid. However, the structure 8-hydroxyoctadec-14-en-10:12-diynoic acid has been suggested by Sehor[27] in a recent publication.

8-Hydroxyoctadec-11-en-9-ynoic acid. An hydroxy acid related to Ximenynic acid (octadec-11-en-9-ynoic acid) has bee isolated from the seed fat of Ximenia caffra sond. It forms about 3-4% of the mixed acids and is probably 8-hydroxyoctadec 11-en-9-ynoic acid[28].

Hydroxypalmitic acid. Bosworth and Helz[29] consider a straight-chain optically active hydroxypalmitic acid to be present in butter fat. The position of the hydroxyl group is not determined.

9:10-Dihydroxystearic acid. An optically active dihydroxystearic acid, m.p. 141° is present in minor amounts (0.5-1.0%) in the mixed acids of castor oil[30]. The hydroxyl groups were shown by Toyama and Ishikawa[31] to be on carbon atoms 9 and 10 and this was confirmed by King[32] who further proved the acid to be one of the optically active forms of the racemic-9:10-dihydroxystearic acid of m.p. 132°.

Hirai and Toyama[33] have obtained a small amount of a 9:10-dihydroxystearic acid from the fatty acids of Lycopodium oil.

Impurolic acid (3:11-dihydroxymyristic acid). Power and Rogerson[34] isolated a small amount of a dihydroxytetradecanoic acid from the seed fat of Ipomea purpurea (South African morning glory) and termed it Impurolic acid. The acid was later isolated by Asahina and Terada[35] from Pharbitis nil Chois, (Japanese morning glory). From oxidative studies, Asahina and Shimidzu[36] proved the acid to be 3:11-dihydroxymyristic acid which has been confirmed by Asahina and Nakanishi[37]. These last workers during their study removed the β-hydroxyl group from the acid and obtained 11-Hydroxymyristic acid, m.p. 53°. Whether or not this acid is identical with a monohydroxymyristic acid,

m.p. 51° obtained by Müller[38] from Angelica archangelica oil is not determined.

9:14-Dihydroxyoctadeca-10:12-dienoic acid. This acid was isolated in very small amounts from tung oil by Davis, Conroy and Shakespeare[39]. It is probably a product of autoxidation of the tung oil rather than a true component of the oil.

Hydroxy Acids Occurring in Waxes:

Wool Wax:* This is a true wax as it consists essentially of th fatty acid esters of alcohols other than glycerol. The wax which is complex in nature is very difficult to analyse and it is, therefore, not surprising that some investigators have fail to identify components isolated by others. As far back as 1892 Lewkowitch[40] noted that the fatty acids of wool wax formed lact nes on heating and increaed in weight on boiling with acetic anhydride. The occurrence of hydroxy acids in wool fatty acids was hence suggested. Darmstaedter and Lifschutz[41] later isolate two hydroxy acids termed lanoceric acid, a dihydroxy acid of composition $C_{30}H_{60}O_4$ and lanopalmic acid which was a mono-hydroxy acid of composition $C_{16}H_{32}O_3$. In addition a large quantity of an acid of m.p. 72-73° and composition $C_{26}H_{52}O_2$ or $C_{27}H_{54}O_2$ was isolated. In 1916 Rohmann[42] reported the occurrence of stearic, cerotic and optically active hydroxy acids in a sample of wool wax. Drummond and Baker[43], however, found no evidence of existence of lanoceric and lanopalmic

* For a review see Quarterly Reviews, 1951, 5, 390.

acids but confirmed the presence of myristic, palmitic, stearic and cerotic acids.

Abraham and Hilditch[44] were the first to suggest that the saturated acids of wool wax are not of normal series but are of substantially unknown structures as the melting points of these saturated acids were lower than the melting points of the corresponding normal acids. They also confirmed that the chief component of wool wax was a solid, m.p. 73-75°, of formula $C_{26}H_{52}O_2$ or $C_{27}H_{54}O_2$ (less probably $C_{25}H_{50}O_2$) but this component was definitely not cerotic acid. A series of acids represented by the formulae $C_{15}H_{30}O_2$, $C_{15}H_{30}O_3$, $C_{20}H_{40}O_2$, $C_{20}H_{40}O_3$ or $C_{20}H_{38}O_3$, $C_{30}H_{60}O_4$ and $C_{30}H_{60}O_3$ or $C_{30}H_{58}O_3$ were also believed to be present. Of these the acids of formulae $C_{15}H_{30}O_3$, $C_{30}H_{60}O_4$ and $C_{30}H_{60}O_3$ or $C_{30}H_{58}O_3$ were considered to be identical respectively with lanopalmic, lanoceric and lanoceric acid lactone described by Darmstaedter and Lifschutz[4] Saturated acids such as myristic, palmitic, stearic, etc., were however, absent. Heiduschka and Nier[45] isolated lanoceric and cerotic acids and claim to have characterised cerotic acid by preparation of known derivatives. Kuwata and Ishii[46], however, confirmed the findings of Abraham and Hilditch[44] that the saturated acids of wool wax were different from normal acids and four saturated acids containing 14, 16, 18 and 20 carbon atoms, not belonging to the normal series were isolated.

In 1938 Kuwata[47] proved lanopalmic acid, m.p. 86-87° to be

an active form of α-hydroxypalmitic acid, by oxidation to 2-ketopalmitic acid, further oxidation of which gave pentadecanoic acid. The latter was also obtained through the oxime.

Results of a very extensive investigation of wool wax fatty acids were published in 1945 by Weitkamp[48] who succeeded in isolating not less than 32 acids. These belonged to four groups: (1) α-hydroxy acids (3.5%), (2) normal saturated acids (7%), iso-acids (22%; these have a terminal iso-propyl group), and anteiso-acids (30%; these have a methyl group on the antepenultimate carbon atom). Of the hydroxy acids only two (2-hydroxymyristic and 2-hydroxypalmitic) acids were identified These differed from the synthetic acids of these structures in being optically active.

Horn and his collaborators[49] have found only recently that wool-wax fatty acids contain nearly 30% of a mixture of the optically active α-hydroxy acids containing 12, 14, 16, and 18 carbon atoms.

Bacterial Waxes: Hydroxy acids of high molecular weight are known to occour in several bacterial waxes. Thus Chargaff[50] separated a hydroxy acid (? $C_{52}H_{104}O_3$) from the wax of Calmette Guérin bacillus. Similarly a hydroxy acid termed leprosinic aci (? $C_{44}H_{88}O_3$) was separated from leprosy and timothy bacterial waxes[51]. Anderson and his co-workers[52] isolated a hydroxy acid ($C_{88}H_{172}O_4$ or $C_{88}H_{176}O_4$) from the waxes human and bovine

bacilli and termed it mycolic acid. The acid, m.p. 54-56° was optically active and contained one hydroxyl and one methoxy group and on pyrolysis gave a volatile acid identified as hexacosanoic acid. The wax from Avian tubercle bacillus was found by Anderson and Crieghton[53] to contain hydroxy acids similar to mycolic acid but having no methoxy group. Two acids (α- and β- mycolic acids) were isolated; the former on pyrolysi gave a branched-chain pentacosanoic acid and the latter gave n-tetracosanoic acid. A third acid (γ-mycolic acid) was later isolated[54] and this on pyrolysis gave a tetracosanoic acid different from normal tetracosanoic acid.

The first suggestion that mycolic acid was not a single acid but a mixture came from Lesuk and Anderson[55], who isolate an acid of composition $C_{104}H_{208}O_3$ from mycolic acid. Confirmatory evidence was provided by Stalleberg-Stenhagen and Stenhagen[56] as they obtained mixtures of closely related acids on pyrolysis of α- and β-mycolic acids. In later work, Lederer and other workers[57] showed that mycolic acids which form about 8% of the waxes from Mycobacterium tuberculosis are branched-chain hydroxy acids derived from n-hexacosanoic acid and of general constitution,

$$CH_3(CH_2)_{23}.\underset{\displaystyle CH(OH).R}{\overset{\displaystyle CH.COOH}{|}}$$

, where R is a comple radical of about $C_{60}H_{121}$-, $C_{60}H_{120}(OH)$- , or $C_{60}H_{120}(OCH_3)$-. In addition two hydroxy acids (i. coryno-mycolic acid and

ii. coryno-mycolenic acid) have been isolated from the lipids of *Corynobacterium diphtheriae*[58].

$$CH_3(CH_2)_{13}.\underset{|}{CH}.COOH \quad CH(OH).(CH_2)_{14}CH_3 \qquad (i)$$

$$CH_3(CH_2)_{13}.\underset{|}{CH}.COOH \quad CH(OH).(CH_2)_7CH{:}CH(CH_2)_5CH_3 \qquad (ii).$$

Other Animal Waxes: Hydroxy acids are present in beeswax. Ikuta[59] has reported the isolation of a hydroxyhexadecanoic acid from beeswax and Findley and Brown[64] state that the fatty acids of beeswax contain about 25% of hydroxy acids.

The oil from castor oil fish, *Ruvettus pretiosus* consists primarily of esters of the type cetyl oleate, oleyl oleate, etc. A monohydroxy-monoethenoid acid of chain-length 18 has been shown to occur in this oil by Cox and Reid[60].

Vegetable Waxes: Hydroxy acids are present in significant amounts in the waxes of conifers combined in the form of etholides. Bougault and Bourdier[61] isolated two hydroxy acids; *sabinic acid* from *Juniperus sabina* wax and *juniperic acid* from several conifer waxes. Sabinic acid was proved[62] to be 12-hydroxydodecanoic acid as oxidation with chromic acid gave dodecanedioic acid and reaction with iodine and phosphorous followed by reduction gave lauric acid. Similarly juniperic

acid was shown to be 16-hydroxyhexadecanoic acid.

Hydroxy acids are present in other waxes such as carnauba wax (60-80%)[63,64], caranda wax (40%)[64], candellila wax (17-48%)[64]. Seven ω-hydroxy acids of chain-length 18, 20, 22, 24, 26, 28 and 30 have been isolated from carnauba wax and identified by Murray and Schoenfeld[65].

A hydroxy acid termed rosilic acid (10-hydroxyoctadecanoic acid) has been claimed to be present in the wax from Drusky rose[66].

The waxes of apple and cranberry cuticles are reported to contain a mono-hydroxy C_{30} acid (ursolic acid)[67].

Hydroxy Acids in Brain and Other Lipids: Apart from the fatty tissue itself, brain is the only bodily organ which contains a very large amount of lipid material. The fatty acids occur as cholesteryl esters, phosphatides, amino phosphatides and as cerebrosides which are glycolipids. Of these, the cerebrosides kerasin and phrenosin are the most important and contain hydroxy acids. Hydroxy acids are also reported to be present in sphingomyelin[68]. Thudichum[69] was the first to do original work upon the chemical nature of the brain and has reported the isolation of a hydroxyoctadecanoic acid, termed neurostearic acid. The acid was later renamed "cerebronic" acid, and was apparently proved by Thierfelder[70] to be a hydroxypentacosanoic acid and later shown to be the α-hydroxy acid by Levene and Jacobs[71]. However, natural cerebronic acid differed from the synthetic racemic- α-hydroxy-n-pentacosanoic acid[72] and the

natural acid was, therefore, considered to be α-hydroxy-iso-pentacosanoic acid.[73] Klenk,[74] however, considered the acid to be α-hydroxytetracosanoic acid as n-tricosanoic acid was obtained on oxidation. Levene and his co-workers[75] later found that cerebronic acid was actually a mixture of hydroxy acids a not a single acid, a finding which is in agreement with that o Grey[76] who succeeded in isolating three hydroxy acids from it. This claim was refuted by Klenk and Diebold[77] who still considered cerebronic acid to be α-hydroxytetracosanoic acid. However, Chibnall, Piper and Williams[78] succeeded in isolating from cerebronic acid three distinct compounds, the C_{22}, C_{24} and C_{26}- α-hydroxy acids. Some time later Müller[79] reported that cerebronic acid was identical with synthetic optically active (dextro-) α-hydroxytetracosanoic acid.

Phrenosinic acid: This acid of formula $C_{25}H_{50}O_3$ has been obtained from 'carnaubon', an alcoholic extract of the lipids of horse and ox kidneys[80].

Hydroxy Acids of Non-Lipid Origin:

Cork: Several hydroxy fatty acids have been found to occur in small proportions in cork. Zetzsche and co-workers[81] isolated three hydroxy acids and termed them phellonic, phloionic and phloionolic acids.

Phellonic acid was originally believed to be a α-hydroxy-behenic acid but was later found to be different from a

synthetic acid prepared by Erlenmeyer and Muller[82]. On the other hand Drake and others[83] considered the acid to be 22-hydroxytetracosanoic acid. The acid was, however, later proved to be 22-hydroxybehenic acid by Guillemonat and Strich[84] and this has been confirmed by Ribas and others.[85]

Phloionic and phloionolic acids were originally considered to be dihydroxy-hexadecamethylene-dicarboxylic acid and trihydroxystearic acid respectively. Zetzsche and Weber[86] later proved them to be 9:10-dihydroxy-1:18-octadecanedioic acid and 9:10:18-trihydroxystearic acid respectively. The latter melts at 104° whereas a synthetic acid prepared by Gensler and Schlein[87] melts at 136-137° and has the threo- configuration. I phloionolic acid has the erythro- configuration it is unusual in having a lower melting point than the threo- isomer. some doubt must, therefore, exist as to the constitution of cork fatty acids.

Recently a hydroxy acid of formula 18-hydroxyoctadec-9-enoic acid has been isolated from cork fatty acids by Ribas and Seoane[88].

Resins: Two hydroxy acids termed convolvulinolic and jalapinolic acids occur in convolvulin resin, a substance obtained from the roots of plants of Convolvulus and Ipomea species (convolvulaceae family).[89] Convolvulinolic acid was initially believed to be 11-hydroxy-13-methyltetradecanoic acid, a structure which was accepted by Power and Rogerson[90].

Asahina and Akasu,[91] however, considered the acid to be 11-hydroxypentadecanoic acid as pentadecanoic acid was obtained on reduction and as the study of the oximes showed a hydroxyl group on carbon 11. Synthetic 11-hydroxypentadecanoic acid prepared by Davies and Adams[92] was different from convolvulinoli acid and hence the structure was re-examined by Kawasaki[93] who proved it to be 11-hydroxytetradecanoic acid identical with the synthetic acid.

Jalapinolic[a] acid first isolated by Mayer[94] in 1855 was considered[95] to be a branched-chain acid of composition $C_{16}H_{32}O_3$, m.p. 67-68° and optically inactive. Power and Rogerson believed it to be $CH_3CH_2.CH(CH_3)CH_2.CHOH.(CH_2)_9COOH$, as oxidation with nitric acid gave sebacic and hexanoic acids; the acid was optically active contrary to the findings of Kromer.[95] Asahina and Yaoi[96] later proved the acid to be 11-hydroxyhexadecanoic acid and this has been confirmed by Kawasaki.[93]

Aleuritic acid (9:10:16-trihydroxyhexadecanoic acid). This is present in shellac and the structure was proved by Nagel and co-workers.[97] Nagel and Mertens[98] converted aleuritic acid into hexadec-9-enoic acid which on oxidations gave 9:10-dihydroxypalmitic acids, m.p. 125° and 89-90°.

A hydroxy acid ($C_{30}H_{48}O_3$) of unknown structure has been reported to occur in the roots of Swertia Japonica.[99]

Ambrettolic acid: Kerschbaum[100] showed that the odoriferous constituent of musk seed oil is a lactone which on hydrolysis

gives 16-hydroxyhexadec-7-enoic acid (ambrettolic acid), later synthesised by Baudart[101]. Two synthetic isomers of ambrettolide have also been reported; iso-ambrettolide (the lactone of 16-hydroxyhexadec-6-enoic acid)[102] and epi-ambrettolide (the lactone of 16-hydroxyhexadec-9-enoic acid)[103]. These too have a musk like odour.

Ustilic acids: 15:16-Dihydroxypalmitic (ustilic acid A) and 2:15:16-trihydroxypalmitic acid (ustilic acid B) are produced by the hydrolytic breakdown of ustilagic acid, a constituent of the Ustilago fungi which attack barley and other cereal grains.

Part IB A New Method of Analysing Oils Containing Epoxy and/or Hydroxy Acids.

Introduction

One problem associated with the study of seed oils is the quantitative determination of the component acids. This is achieved by converting the mixed acids resulting on hydrolysis (usually a complex mixture) into a large number of fractions each simple enough to be characterised by the iodine value, saponification equivalent, etc.. The conversion of the initiall complex mixture into a number of simple fractions requires a number of separate techniques of which low-temperature crystallisation and fractional distillation are now the most frequently used. The former separates acids mainly on the basis of the degree of unsaturation; the latter separates esters mainly on the basis of chain-length.

The above procedure though satisfactory for most of the seed oils is unsatisfactory for oils containing hydroxy or epoxy acids. Attempts by Gupta, Hilditch and Riley,[30] to separate ricinoleic acid from other unsaturated acids of castor oil by low-temperature crystallisation from solvents were unsuccessful. Determination of ricinoleic acid as hydroxystearic acid (after hydrogenation) gave unsatisfactory results and the oil was ultimately analysed without subjecting the mixed acids to low-temperature crystallisation or fractional distillation. The ricinoleic acid content was calculated from the acetyl value determined by Riley's[105] method after making allowance for the dihydroxystearic acid obtained by crystallisation. The

content of linoleic acid was determined from the ultra-violet absorption after alkali isomerisation, whilst oleic acid was calculated from the iodine value after making allowance for the linoleic and ricinoleic acids; the content of saturated acids was determined as a group. This method is obviously dependant on satisfactory determination of iodine value.

Throughout his work on Strophanthus sarmentosus seed oil, Gunstone[15] encountered difficulty in determining the iodine value of fractions containing the hydroxy acid. [No difficulty was met with in the low-temperature crystallisation, probably due to the low content of the hydroxy acid]. The iodine values were higher than expected and less concordant than usual. This effect was reduced or eliminated by acetylation of the esters and in the Strophanthus oils examined,[15] fractions containing the hydroxy acid were methylated and acetylated before distillation and the proportion of methyl acetoxyoctadecenoate was computed solely from the saponification equivalent. This method is dependent on the thermal stability of the acetoxy ester and it is perhaps on account of this factor that Gupta, Hilditch and Riley[30] did not distil their esters.

Vidyarthi and Mallya[2] have made similar remarks on the unsatisfactory iodine value of Vernonia anthelmintica seed oil considered by them to contain 11-hydroxyoctadec-9-enoic acid, but later proved by Gunstone[1] to be 12:13-cis-epoxyoleic acid.

Another method of examining mixtures containing ricinolei

acid has been described by Achaya and Saletore.[106] Saturated acids were first removed by lead salt separation and the remaining acids were then separated into two fractions by a technique in which oleic acid and a little linoleic acid formed a urea complex whilst ricinoleic and most of the linoleic acid did not. This last fraction was subsequently methylated, acetylated and distilled.

The normal procedure needed yet further modification for the examination of Vernonia anthelmintica seed oil shown to contain 12:13-epoxyoleic acid by Gunstone[1]. The mixed acids could not be easily esterified by the usual methods as the epoxide reacted readily with the reagents. This difficulty was avoided by converting the epoxy acid to the corresponding dihydroxy acid by treatment with acetic acid followed by alkali hydrolysis. The dihydroxy acid was then separated from the non-hydroxy acids by partition between 80% methanol and light petroleum (b.p. 40-60), the non-hydroxy acids being concentrated in the latter. The non-hydroxy acids were subsequently esterifi and distilled and the composition of the distilled fractions computed in the usual way. The fraction in which the dihydroxy acid was concentrated was methylated and acetylated and the content of dihydroxy acid calculated from the saponification equivalent before and after acetylation. The small amount of non-hydroxy acids in this fraction was assumed to have the same composition as that of the distilled fraction. An approximate

composition of the oil was thus determined.

In the persent work a method has been developed for analysing oils containing epoxy-, mono- or dihydroxy acids based largely on the method just described and depending on the separation of hydroxy from non-hydroxy acids by partition between suitable solvents.

After the present work was concluded, a paper was published by Sreenivasan et.al.[107] on the analysis of castor oil. The methyl esters were resolved quantitatively into hydroxy and nom-hydroxy esters by reaction with succinic anhydride in toluene The composition was then calculated from the weight of each fraction (ricinoleic), the ultra-violet absorption after alkali isomerisation (linoleic), the iodine value (oleic + saturated), and from periodate oxidation (dihydroxystearic). Surprisingly the non-hydroxy fraction showed conjugated unsaturation and accordingly these workers used the Woburn procedure for determining iodine value. In view of our experience with the glycol value (see page 102) we do not consider this method to be better than that of Riley[105] as Sreenivasan et. al. claim.

Method of Analysis

Preparation of Mixed Hydroxy Acids Free of Unsaponifiable Material:

Oils which do not contain any epoxy acid are hydrolysed directly by refluxing with ethanolic potassium hydroxide solution for 1-2 hours. Generally 150-200 g. of the oil are hydrolysed with 75-100 g. of potassium hydroxide in 750-1000 ml of ethanol. When the oil contains an epoxy acid, this is converted to the corresponding acetoxy-hydroxy acid prior to the alkaline hydrolysis, by refluxing the oil with five volumes of glacial acetic acid for 5-7 hours. After distilling off the acetic acid (the last traces under vacuum), the product is saponified with alcoholic potassium hydroxide solution as above. After hydrolysis about half of the alcohol is distilled off and the product diluted with water. The unsaponifiable material is then removed by extraction with ether (4-5 times with 500 ml. per 150-200 g. of fat). The combined ether extracts are washed with water (2-3 times with 200 ml) and the washings are added to the extracted soap solution which is then acidified with 25% sulphuric acid. The fatty acids so liberated are extracted with ether, the ether solution is washed free of mineral acid and dried over sodium sulphate. Removal of the solvent affords the mixed hydroxy acids.

Separation of Hydroxy from Non-Hydroxy Acids:

The mixed acids obtained from above are separated into two fractions by partition between light petroleum (b.p. 40-60°) and

methanol-water (4:1) which have been previously equilibriated by shaking together. When the mixed acids are rich in hydroxy acids they are dissolved in 80% methanol and extracted with light petroleum whilst acids rich in non-hydroxy acids are dissolved in light petroleum and extracted with 80% methanol. I this way two fractions are obtained:

(i) Methanol fraction rich in hydroxy acids (fraction C).

(ii) Petroleum fraction rich in non-hydroxy acids (fractions A+

The procedure is best illustrated by an actual example.

To 1 litre of 80% methanol in each of three separatory funnels (1-3) was added about 50 g. of acids from Vernonia anthelmintica seed oil; 500 ml. of 80% methanol was placed in each of two further funnels (4-5). Light petroleum (250 ml.) wa added to the first funnel and after equilibrium passed to each of the other four funnels in turn. This was followed by other portions of light petroleum until little or no material was extracted from the methanol solutions. Acids remaining in the methanol solutions were then recovered by distilling most of th methanol, adding water and extracting with ether. The distribut of the material was as follows:

	Methanol solutions.			Light petroleum extracts.				
No.	1	2	3	4	5	5	4	3	2	1
Wt., g.	113.8			4.3		1.1	1.8	3.6	9.9	21.3

These figures suggest that the separation of dihydroxy aci

acid from non-hydroxy acids is sharp and readily effected. Similar results were obtained with Strophanthus hispidus seed oil containing only a small proportion of a mono-hydroxy acid (see page 73).

Separation of Saturated from Unsaturated Acids:

The non-hydroxy acids (petroleum extracts from above) are separated into saturated and unsaturated acids by crystallisati from methanol (10ml. per gram of acids) at -20°C. The acids dissolved in methanol are placed in a constant temperature bath consisting of a Dewar flask containing about 6 litres of alcoho cooled to the desired temperature with solid carbon dioxide. Th flask is left in the bath overnight and the solids obtained by filtration on a Buchner funnel. The funnel is maintained at the temperature of crystallisation by surrounding it with cold alcohol contained in a suitable jacket encasing the funnel. The solids are sucked dry and then washed twice with a little cold solvent, the solvent being cooled 2-3°C below the cryatallisati temperature. The solids are then removed from the funnel by was ing with hot methanol and removal of the solvent gives the saturated acid fraction A. From the mother liquor the unsaturate acids are recovered by removal of the solvent. The original mixed acids are thus divided into three fractions in which the saturated (A), unsaturated (B) and the hydroxy acids (C) are separately concentrated.

Examination of Fractions A, B, and C:

Esterification: The saturated acids (A) are dissolved in

methanol (5 ml./g.), concentrated sulphuric acid added (1 ml. per 100 ml. of methanol) and the solution is refluxed for 1 hou About one third of the methanol is then removed, the product diluted with water and extracted with ether, the ether solution being washed 3 times with 10% potassium hydroxide solution to remove unesterified acids and then with water until free of alakli. The esters are obtained on removing the ether after drying the etheral solution over sodium sulphate. The more unsaturated fractions B and C are esterified by dissolving in methanol (5 ml./g.), passing dry hydrogen chloride (1 g. per 100 of methanol) and leaving the solution overnight. After removal of some of the methanol at about 40° under reduced pressure, the esters are extracted and worked up as before.

Acetylation: The esters of fractions B and C are acetylated by refluxing for 2-3 hours with acetic anhydride (5 ml./g.), exces acetic anhydride being then decomposed by boiling with water fo 1/2 to 1 hour, (same volume as acetic anhydride). The mixture is then diluted with plenty of water and extracted with ether. The ether extract is washed free of acetic acid, dried over sodium sulphate and the removal of ether then gives the acetylated produc

Fractional Distillation of Methyl Esters: Fractions A and B are distilled through an electrically heated and packed column unde reduced pressure (0.2-0.5 mm. Hg), the column available being a Towers column packed with glass helices, When fraction A is small, a small Widmer column is used for the distillation.

Fractions of about 2-3 g. are collected in a multiple receiver. From the iodine value and saponification equivalent of each fraction and the spectroscopic examination after alkali isomerisation of selected fractions, the composition of each ester fraction is calculated.

Fraction C is not distilled but its content of hydroxy acid calculated from the saponification equivalent of the ester before and after acetylation. The non-hydroxy acids in fraction C are considered to have the same composition as the acids in factions A and B together. The content of hydroxy acids in frac C has always been greater than 90% and this assumption is, therefore, not considered to introduce a large error into the final result.

Discussion:

The method of analysis has been successfully applied to V. anthelmintica seed oil containing over 70% of epoxyoleic acid, to S. hispidus seed oil which contains less than 20% of a monohydroxyoctadecenoic acid, to ergot oil containing about 35% of ricinoleic acid and to Cephalocroton cordofanus seed oil which has now been shown to contain epoxyoleic acid in high proportion. The method may be usefully applied to oils containing epoxy, monohydroxy or dihydroxy acids and, whilst there is a little more manipulation than in low-temperature crystallisation, no special apparatus or materials are required.

Method of Calculation.

It is difficult to generalise the method of calculation as each fraction has to be carefully considered on the basis of its iodine value, saponification equivalent and absorption spectra after alkali isomerisation. The corresponding values for adjacent fractions may also have to be considered.

The method employed for deriving the composition of fractions A and B is similar to that of Hilditch[108] and fraction C has been calculated from the saponification equivale of esters before and after acetylation. Examples of each are given below.

It is convenient at this stage to indicate the convention used to describe the fatty acids and their methyl esters. Esters of acids containing 18 carbon atoms are referred to as C_{18} esters and C_{18} esters of mono-, di-, and trienoic acids are named C'_{18}, C''_{18}, and C'''_{18} esters respectively. Saturated acids or esters are given the superscript °; thus palmitic acid is C°_{16} acid. $"C_{18}"$ or $"C_{16}"$ indicate a mixture of C_{18} or C_{16} acids or esters.

Fraction A.

A little oleic acid accompanied by smaller amounts of hexa- and tetradecenoic acids are known to be present in the most saturated group of acids. Hence myristic-palmitic or palmitic-stearic esters may also contain esters of monoethenoic esters of the same chain-length.

The amount of mono-ethenoid ester is derived from the iodine value of the fraction and the composition of the remaining saturated binary mixture is calculated from the saponification equivalent, after making allowance for the unsaturated ester.

Example: Ergot Oil, Table 26, page 93.

Fractions A_2 to A_6 are very similar in saponification equivalents whilst the iodine values tend to drop from A2 to A6. These are probably mixtures of C°_{16} and C'_{16} esters as the saponification equivalents of pure C°_{16} and C'_{16} esters are 270.4 and 268.4 respectively. The variation in iodine value is probably caused by the changing proportion of C'_{16} ester. The saponification equivalent of A1 suggests the presence of C_{14} and C_{16} esters, the small iodine value being due to a small proportion of mono-ethenoid ester. This is probably C'_{16} as it is present in fractions A2 to A6. The iodine values increas from fractions A7 to A9 and these in combination with increasi saponification equivalents suggest the presence of C°_{16} and " C''_{18} esters, the unsaturated ester present being C'_{18}. No C'_{16} ester is likely to be present as the amount of it as continuously dropped from A2 to a minimum value in A6.

A2. Wt. 2.45 g; iodine value 2.1; sap. equiv. 268.7

$$\text{Wt. of } C'_{16} \text{ ester} = \frac{\text{wt. of fraction X iodine value}}{\text{iodine value of pure } C^{\circ}_{16} \text{ ester.}}$$

$$= \frac{2.45 \times 2.1}{94.6} = 0.05 \text{ g.}$$

Hence A2 contains 2.40 g. C°_{16} and 0.05 g. $C^{'}_{16}$ ester.

A9. Wt. 2.93 g; iodine value 34.4 ; sap. equiv. 287.5

Weight of $C^{'}_{18}$ ester = $\frac{\text{wt. of fraction} \times \text{iodine value}}{\text{iodine value of pure } C^{'}_{18} \text{ ester.}}$

$$= \frac{2.93 \times 34.4}{85.6}$$

$$= 1.18 \text{ g.}$$

Hence saturated esters = 2.93 - 1.18 = 1.75 g.

If x is the S.E. of the saturated esters, it follows from the definition of saponification equivalent that

$$\frac{2.93}{287.5} = \frac{1.75}{x} + \frac{1.18}{\text{S. E. of } C^{'}_{18} \text{ ester.}}$$

$$= \frac{1.75}{x} + \frac{1.18}{298.5}$$

Hence x = 281.8 . This suggests that the saturated part of the fraction A9 is a mixture of C°_{16} and C°_{18} esters as the saponification equivalents of these pure esters are 270.4 and 298.5, respectively.

If y is % wt. of C°_{16} ester in the binary saturated mixture, then

$$\frac{y}{\text{S.E. of } C^{\circ}_{16} \text{ ester}} + \frac{100 - y}{\text{S.E. of } C^{\circ}_{18} \text{ ester}} = \frac{100}{281.8}$$

Hence y=57.2%

This gives A9 as C°_{16} = 1.00g; C°_{18} = 0.75g; $C^{'}_{18}$ = 1.18 g.

Fraction B:

This will consist mainly of the esters of unsaturated acids of the C_{16}, C_{18} and C_{20} series. Small amounts of esters of saturated acids like myristic, palmitic or stearic may also be present together with esters of mono- or diacetoxy fatty acids. Esters of C_{16} or lower series will distil first followed by C_{18} and C_{20} series.

Example: Cephalocroton cordofanus Seed Oil, Table 12, p. 6

Fractions B3 - B9 are very similar in saponification equivalent whilst the iodine value shows a tendency to fall. These are probably C_{18} mixtures the variation in iodine value being caused by changing proportions of the various C_{18} esters. B6 being about the middle fraction was chosen for spectroscopic examination after alkali isomerisation.‡

B6. Weight. 3.76 g; iodine value 143.9; Sap. equiv. 393.1

$E^{1\%}_{1cm.}$ at 234 mμ	before isomerisation	Nil.
$E^{1\%}_{1cm.}$ at 268 mμ	before isomerisation	Nil.
$E^{1\%}_{1cm.}$ at 234 mμ	after isomerisation	598.0
$E^{1\%}_{1cm.}$ at 268 mμ	after isomerisation	5.5

C^{III}_{18} is considered to be absent on account of the very low absorption value ($E^{1\%}_{1cm}$) at 268 mμ.

‡ Spectroscopic determinations were done on acids.

Considering B6 as consisting only of C_{18} esters (from S.E.), from the spectral value $\% C''_{18} = \frac{598 \times 100}{906} = 66.0$

Since the iodine value of pure C''_{18} ester is 173.2, the contribution of 66% of C''_{18} ester to the iodine value of the fraction is

$$\frac{66 \times 173.2}{100} = 114.3$$

Therefore, $\% C'_{18}$ ester $= \frac{\text{I.V. of fraction} - \text{I.V. due to } C''_{18}}{\text{I.V. of pure } C'_{18} \text{ ester}}$

$$= \frac{142.9 - 114.3}{85.6} = 33.4$$

Therefore $C'_{18} + C''_{18} = 66.0 + 33.4 = 99.4$ %

In view of the experimental error involved in the determination of ultra-violet absorption this is taken to show that this fraction contains only $C'_{18} + C''_{18}$ esters. The compositions of fractions B3 - B9 can now be calculated from the iodine value alone as follows:

B3. Weight 2.86g; iodine value 143.2

Wt. (g.) of C''_{18} ester $= \left(\frac{\text{I.V. of fraction} - 85.6}{173.2 - 85.6}\right) \times \text{Wei}$

$$= \left(\frac{143.2 - 85.6}{173.2 - 85.6}\right) \times 2.86$$

$$= 1.88 \text{ g. or } 65.7 \%.$$

Hence C'_{18} ester $= 2.86 - 1.88 = 0.98$ g or 34.3%

B1. The S.E. of B1 (and B2) indicates that this fraction contains some esters lower than C_{18}. If it is assumed to be

$^{"}C^{"}_{16}$ and $^{"}C^{"}_{18}$ esters, then the amounts of these can be computed from the iodine value or the saponification equivalent of the fraction. Using the latter it can be shown that the C_{16} is entirely saturated and we have calculated the composition of this fraction as a mixture of C°_{16} and $^{"}C^{"}_{18}$ esters (as in B3) on the basis of its iodine value.

Weight 2.84g; iodine value 101.3; Sap. equiv. 289.3

$$\% \ ^{"}C^{"}_{18} \text{ esters} = \frac{100 \times \text{I.V. of fraction}}{\text{I.V. of fraction B3}}$$

$$= \frac{100 \times 101.3}{143.2} = 70.7 \% \text{ or } 2.01 \text{ g.}$$

Hence C°_{16} = 29.3 % or 0.83 g.

From B3 it is known that C'_{18} and C''_{18} are 34.3 % and 65.7 %, respectively. Hence 2.01 g. of $^{"}C^{"}_{18}$ esters are distributed accordingly.

This gives fraction B1 as C°_{16}=0.83 g, C'_{18}= 0.69 g., C''_{18} = 1.3

B11. The low saponification equivalent of this fraction (and B10) suggests the presence of acetylated esters. This fraction is hence computed as $^{"}C^{"}_{18}$ esters (same as in fraction B9 of I.V. 138.1, SE. 294.4) and diacetoxy-C'_{18} ester

Weight. 3.27g; Sap. equiv. 245.4; unsaponifiable 0.77 g.

The Sap. equiv. corrected for unsaponifiable material

$$= \frac{2.50 \times 245.4}{3.27} = 187.6$$

If X is the % "C_{18}" esters, then

$$\frac{X}{\text{S.E. of X}} + \frac{100 - X}{\text{S.E. diacetoxy-}C_{18}\text{ ester}} = \frac{100}{\text{S.E. of fraction.}}$$

$$\frac{X}{294.4} + \frac{100 - X}{137.5} = \frac{100}{187.6}$$

Hence X = 49.9% or 1.25 g.

Therefore, weight of diacetoxy ester = 2.50 - 1.25 = 1.25 g.

From B9 it is found that "C_{18}" esters contain 39.9% C'_{18} and 60.1% C''_{18}.

Hence 1.25 g. of "C_{18}" esters contain 0.50g. of C'_{18} and 0.75 g. of C''_{18}.

Thus fraction B11 is C'_{18} = 0.50 g., C''_{18} = 0.75 g., diacetoxy-C'_{18} ester = 1.25 g., unsaponifiable material = 0.77 g..

Fraction C:

This will consist mainly of the acetylated esters of mono- or dihydroxy acids, together with smaller amounts of esters of non-hydroxy acids and unsaponifiable material. The amount of hydroxylated acids is calculated from the saponification equivalent of the esters before and after acetylation and the non-hydroxy acids are assumed to have the same composition as that of the non-hydroxy acids in fractions A and B together, excluding unsaponifiable material. The formula used for the

calculation of the hydroxylated acids is as follows:

$$\% \text{ Hydroxy ester} = \frac{100 \times M(B - A)}{56100n - B(M_1 - M)} \quad \text{where,}$$

M and M_1 are the molecular weights of the hydroxy and acetoxy-esters; n is the number of hydroxyl groups present in the este A and B are the observed saponification values of the esters before and after acetylation. The % ester is then converted to the % acid as shown in the example following:

Example: Cephalocroton cordofanus seed oil, Table 13, p. 62

C acids = 70.0 % of the mixed acids.

Unsaponifiable material in C acids = 0.43 %.

S.E. of esters before acetylation = 326.1

S.E. of esters after acetylation = 141.5

Therefore, dihydroxy-C'_{18} ester in mixed esters = 93.5 %.

Hence, other esters + unsaponifiable material = 6.5 %.

The saponification equivalent (X) of other esters plus the unsaponifiable material may be calculated to be 295.2 from the equation:

$$\frac{93.5}{\text{S.E. dihydroxy-}C'_{18}\text{ ester}} + \frac{6.5}{X} = \frac{100}{326.1}$$

Now process:

	Dihydroxy-C'_{18}	Others.	Total.	
Esters (% wt.)	93.5	6.5	100	(i)
Sap. equivalent.	328.5	295.2	326.1	(ii)
$\frac{\text{(i) X S.E. Acid.}}{\text{S.E. ester.}}$	89.52	6.19	95.71	(iii)
* Acids (% wt.).	93.5	6.5	100.0	

* % Acids is calculated from $\frac{\text{(iii) X 100}}{95.71}$

Since fraction is 70.0% of the mixed acids, it will contribute, 65.45 g. of dihydroxyoctadecenoic acid, 0.30 g. unsaponifiable material and 4.25g. of non-hydroxy acids per 100 g. of the mixed acids.

The composition of non-hydroxy acids (4.25g.) is then calculated as 0.48 g. C°_{16}, 0.34g. C°_{18}, 0.09 g. $C20^{\circ}$, 1.21 g. C'_{18} and 2.13 g. C''_{18}, from the composition of the non-hydroxy acids in fractions A and B together excluding unsaponifiable material.

Part IC. Quantitative Determination of the Component Acids of Some Seed Oils (Vernonia anthelmintica, Cephalocroton cordofanus and Strophanthus hispidus) and Ergot Oil.

Vernonia anthelmintica Seed Oil.

Introduction:

Vernonia anthelmintica (Willd), syn. Serratula anthelmintica (Roxb.) and Conyza anthelmintica (Linn.), also known as the purple flea-bane, belongs to the family Composita

The plant is a tall, robust leafy annual and is met with throughout India to Ceylon and Malacca, ascending to 5500 ft. in the Himalayas and Khasi mountains. The seeds are bullet-shaped with pale longitudinal ridges, greenish brown in colour 1/8 to 1/4 inch long and taste bitter. They are reported[109] to possess strong anthelmintic and diuretic properties, are used for the treatment of skin diseases, and as an ingradient of a powder used on snake bites. They are also said to preserve woolen goods from the attack of insects. The bitter principle in the seeds is stated[110] to have a weak vermifugal action on ascaris, a somewhat stronger action on oxyuris, but to have no action on hookworm and tapeworm.

The seed fat was first analysed by Kesava-Menon[109] who quotes:

> sap. value 202.9, iodine value 71.0, unsaponifiable material 1.79%, fatty acids 91.6% of iodine value 73.4, neutralisation value 195.1 and mean molecular weight 287.4

Based on the high acetyl value of the oil, Vidyarthi and Mallya[2] demonstrated the occurrence of a hydroxy acid and

considered it to be an isomer of ricinoleic acid. The mixed acids with an acetyl value 118.2, $[\alpha]_D^{28}$ - 7.2° contained 60% of a hydroxy acid $C_{18}H_{34}O_3$, saponification equivalent 299.0, iodine vale 108.4 and $[\alpha]_D^{28}$-7.8°. The oil had an acetyl value 135.1 and $[\alpha]_D^{28}$-10.7°.

The high acetyl value and the optical rotation of the seed fat were later confirmed by Majumdar[111] who gives:

n_{32} 1.4860, $[\alpha]_D^{30}$9.8°, d_{30} 0.9050, saponification value 175.5, acid value 51.3, iodine value 54.6, unsaponifiable material 1.68% and acetyl value 106.7 .

Complete analysis of the oil was first recorded by Vidyarthi[2] in 1945. From the fractional distillation of the acetylated methyl esters, the composition of the oil was calculated as:

resin acids (2%), myristic (7.4%), palmitic (7.0%), stearic (5.9%), oleic (5.7%), linoleic (9.6%) and vernolic acid (62.4%).

From the potassium permanganate - acetone oxidation of methyl vernolate, azelaic acid, m.p. 108° and α-hydroxynonanoic acid, m.p. 70° were isolated. Vernolic acid was hence assigned the structure 11-hydroxyoctadec-9-enoic acid. The isolation of α-hydroxynonanoic acid is rather difficult to visualise as further oxidation to octanoic acid should have proceeded under the conditions of oxidation.

In 1954, Gunstone[1] proved vernolic acid to be 12:13-epoxyoctadec-9-enoic acid and thus for the first time an epoxy

acid was shown to occur naturally in a fat. Brief details of his work are as follows:

Certain difficulties were encountered during the investigation of the oil. The mixed fatty acids could not be easily methylated with methanolic hydrogen chloride and the product when obtained had a low saponification equivalent. Methanol containing concentrated sulphuric acid gave a product with a high equivalent This suggested the presence of an epoxide which was shown to occur in high proportions by King's[112] procedure.

Because of the reactivity of the epoxy group it was converted to the corresponding dihydroxy acid by treatment with acetic acid prior to the usual alkaline hydrolysis. A concentrate of the dihydroxy acid was then easily obtained by partition of the mixed acids between 80% methanol and light petroleum (b.p. 40-60°) and a pure sample , m.p. 53-54° was obtained by subsequent crystallisation from ether - light petroleum (b.p. 40-60°). The [113] α- glycol, hydrogenated and then oxidised with periodic acid gave hexaldehyde and 11-formylundecanoic acid; the latter on further oxidation with potassium permanganate in acid solution gave dodecanedioic acid, m.p. 124-126°, showing that the hydroxyl groups were on carbon atoms 12 and 13. Oxidation of the unsaturated dihydroxy acid with potassium permanganate in acetic acid[114] gave hexanoic acid and azelaic acid indicating that the double bond occupies the 9:10 position. This was certainly the cis- isomer and after isomerisation, the higher melting trans- isomer, m.p. 67.5-69.5° was obtained. Vernolic acid was hence

proved to be 12:13-epoxyoctadec-cis-9-enoic acid . An approximate analysis of the oil was also carried out (method has been described on page 22) and gave the following:

myristic (0.5%), palmitic (3.5%), stearic (1.5%), oleic (6.0%), linoleic (16.5%) and epoxyoleic acid (72.0%) by weight.

The structure of vernolic acid has been confirmed by Raman[3].

The present investigation was undertaken to obtain a more accurate analysis of this seed oil by the new method already described on page 24. Full experimental details are given on the pages following.

Experimental:

Quantitative Determination of Component Acids:

The oil was extracted with light petroleum (b.p. 40-60°), from a further sample of the seeds previously used[1] in the characterization of the epoxy acid. The mixed acids containing the dihydroxyoleic acid (from the original epoxyoleic acid by acetic acid - alkali treatment) were obtained by the method already described (page 24) and were separated into two fractio by partition between 80% methanol and light petroleum (b.p. 40-60°). The non-hydroxy acids (petroleum extract) were crystallise from methanol at -20°C , giving fractions A (insoluble) and B (soluble). At this stage some difficulty was encountered beca of the unsaponifiable material present. Fraction A should consist mainly of saturated acids but had a high iodine value (79) which dropped (24.4) on removal of the unsaponifiable material. That of fraction B fell from 163.1 to 149.0 when similarly treated. The unsaponifiable material in fraction C (hydroxy acid fraction) was determined quantitatively[115].

Fraction A was esterified with methanol and sulphuric acid and fractions B and C with methanolic hydrogen chloride. The whole of fraction B and a part of fraction C were subsequently acetylated. Fractions A abd B were then distilled, the small quantity of A esters being distilled through a small Widmer column, and the composition of these ester fractions was computed in the usual way, (page 29). Fraction C was

calculated in terms of dihydroxyoleic acid, unsaponifiable material and non-hydroxy acids having the same composition as in fractions A and B together. The content of dihydroxyoleic was calculated from the saponification equivalent of the esters determined in quadruplicate, before and after acetylation.

The absence of a monohydroxy acid was indicated from the following experiment:

A sample of fraction C acids was dissolved in 80% methanol and extracted with light petroleum (b.p. 40-60°) for 16 hours. The petroleum extract (5.8%) contained 56% of dihydroxyoleic ac as determined by the glycol value(page 102) and 49% as determine by the saponification equivalent (single determination) of the esters before and after acetylation. Since the amount of hydrox acid present in the fraction was all accounted for as α-glycol, no monohydroxy acid was present in this extract where such an acid would be expected to concentrate.

The results of the analysis are reported in the following tables.

Table 1. Characteristics of V. anthelmintica Seed Oil.

	Oil.	Mixed hydroxy acids.[a]
Saponification equivalent.	320.7	329.5
Iodine value.	101.7	107.4
Epoxy glyceride (% wt.).	71.5	-
Absorption $E^{1\%}_{1cm}$ at 234 mμ, (180°/60min.).	-	115.3 [b]

a. Mixed hydroxy acids: This refers to the mixed acids prepared after conversion of the epoxy acid to the corresponding dihydroxy acid.

b. Measured on acids free of unsaponifiable material.

Table 2. Partition of Mixed Hydroxy Acids Between 80% Methanol and Light Petroleum (b.p. 40-60°).

To 1 litre of 80% methanol in each of the three separator funnels (1-3) was added about 50 g. of the mixed hydroxy acids and 500 ml. of the same solvent was placed in funnels 4 and 5. Light petroleum (250 ml.) was added to the first funnel and after equilibrium passed to each of the other four funnels in turn. This was followed by other portions of light petroleum until little or no material was extracted from the methanol solutions. The distribution of the material was as follows:

	Methanol solutions.					Petroleum Extracts.				
No.	1	2	3	4	5	5	4	3	2	1
Wt., g.	113.8			4.3		1.1	1.8	3.6	9.9	21.3
	118.1 (C),					37.7 (A + B)				

Table 3. Low-Temperature Crystallisation of Non-Hydroxy Acids

The non-hydroxy acids (37.7 g.) were crystallised from methanol (380 ml.) at -20° C and the unsaponifiable material removed subsequently from the two fractions thus obtained.

Fraction.		Wt. (g.)	% (Wt.)	Iodine Value
A	Acids insoluble at -20° C.	5.9	3.8	24.4
	Unsaponifiable material.	8.1	5.2	-
B	Acids soluble at -20° C.	21.1	13.5	149.0
	Unsaponifiable material	2.6	1.7	-
C	Methanol extract.	118.1	75.8	88.9

Table 4. **Distillation Data - Fraction A.**

No.	Wt., g.	I.V.	S.E.	C°_{16}	C°_{18}	C°_{20}	C'_{18}	N.S
A1	2.29	13.1	276.9	1.70	0.24	-	0.35	-
A2	1.76	20.7	286.8	0.66	0.67	-	0.43	-
A3*	0.66	-	361.2	-	-	0.59	-	0.(
T.	4.71			2.36	0.91	0.59	0.78	0.(
		% Esters.		50.1	19.3	12.5	16.6	1.!
		% Acids.		49.9	19.3	12.6	16.6	1.(
		Increment (3.8%)		1.90	0.73	0.48	0.63	0.(

* This fraction contains 0.066 g. of unsaponifiable material.

Table 5. Distillation Data - Fraction B.

No	Wt., g.	I.V.	S.E.	C°_{16}	C°_{18}	C°_{20}	C'_{18}	C''_{18}	C'_{18} diOAc	N.S.
B1	2.68	112.4	280.7	0.69	0.08	-	0.34	1.57	-	-
B2	2.58	150.1	291.6							
B3	2.47	153.4	292.3							
B4	2.73	152.1	290.9							
†B5	2.76	150.1	292.9	-	0.58	-	2.46	11.51	-	-
B6	2.66	146.5	292.3							
B7	1.35	141.5	293.9							
‡B8	1.91	102.6	298.8	-	0.06	-	0.27	1.28	0.13	0.17
T	19.14			0.69	0.72	-	3.07	14.36	0.13	0.17
		% Esters.		3.61	3.76	-	16.04	75.02	0.68	0.89
		% Acids.		3.60	3.76	-	16.06	75.09	0.55*	0.94
		Increment (13.5%)		0.49	0.51	-	2.17	10.14	0.07*	0.12
Acids in fraction A.				1.90	0.73	0.48	0.63	-	-	0.06
Acids in A + B.				2.39	1.24	0.48	2.80	10.14	0.07*	0.18
% Acids in A + B, excluding unsaponifiable material, and dihydroxyoleic.				14.02	7.27	2.82	16.42	59.47	-	-

* Calculated as dihydroxyoleic acid.

‡ 0.944 g. of this fraction contains 0.083 g. of unsaponifiabl

†B5 Acids: iodine value 158.3, $E^{1\%}_{1cm.}$ (180°/60min.) at 234 mμ 71

" , " at 268 mμ 1.

Table 6. **Fraction C.**

Saponification equivalent before acetylation	326.1
Saponification equivalent after acetylation.	143.4
Unsaponifiable material in esters.	1.4 %.

Composition of C (% wt.):	Dihydroxyoleic acid.	90.8
	Non-hydroxy acids.	7.8
	Unsaponifiable.	1.4

The non-hydroxy acids in fraction C are assumed to have the same composition as that of fractions A and B together without dihydroxyoleic acid and unsaponifiable material. Hence the composition of C works out as follows:

	C°_{16}	C°_{18}	C°_{20}	C'_{18}	C''_{18}	Dihydroxy oleic	N.S
C acids.	0.83	0.43	0.17	0.97	3.51	68.83	1.0
[Increment, (75.8%)].							

Table 7. Component Acids of V. anthelmintica Seed Oil.

Acid.	A	B	C	Unsaponifiable.	Total.	Excluding unsaponifiable.		
						%(Wt)	%(Mol)	%(W
Palmitic	1.90	0.49	0.83	-	3.22	3.5	4.2	3.
Stearic	0.73	0.51	0.43	-	1.67	1.8	2.0	1.
Arachidic	0.48	-	0. 17	-	0.65	0.7	0.7	0.
Oleic	0.63	2.17	0.97	-	3.77	4.1	4.4	4.
Linoleic	-	10.14	3.51	-	13.65	14.9	16.1	15.
Dihydroxy-oleic	-	0.07	68.83	-	68.90	75.0	73.6	-
Epoxyoleic	-	-	-	-	-	-	-	73
Unsaponi-fiable.	0.06	0.12	1.06	6.90	8.14	-	-	-

* Since the quantity of epoxyoleic glyceride determined directly (74.1 % mol.) exceeds the quantity of dihydroxyoleic acid (73.6 % mol.), all the latter is considered to have been originally present as epoxyoleic glyceride. This final column gives the composition of the original acids on a weight basis.

Identification of Individual Acids:

The presence of all the acids listed in Table 7 has been previously confirmed[1] with the exception of arachidic and oleic acids.

The oleic acid present in fractions A1 and A2 has now bee identified by oxidation to 9:10-dihydroxystearic acid, m.p. and mixed melting point, 129-130°.

Discussion:

The present analysis shows good agreement with the approximate analysis reported by Gunstone[1], Vidyarthi[2], however has quoted a higher content of saturated acids (20.3%) with corresponding lower amounts of linoleic and vernolic acids.

Cephalocroton cordofanus Seed Oil.

Introduction:

Cephalocroton cordofanus (Hochst) belongs to the family Euphorbiaceae. The plant, a bushy shrub about 2 feet high and seeding scantily is reported[4] to be thinly scattered over a large portion of Sudan. The fruit is covered with minute star-shaped hairs, is rough to touch, and consists of a hard brown capsule, about 2/3 inch wide and somewhat triangular in cross-section as it is three-lobbed. The seeds fall out when the fruits dry. The seeds known locally as Dingilli, consist of a smooth, light brown hard husk (24% of the seed) and a soft white kernel. The seeds are not poisonous as they are eaten by the natives without any ill effects. A pale yellow oil of pleasant taste and odour is obtained on extraction of the seed with light petroleum.

Study of the seed oil has been confined solely to the work of Henry and Grindley[4] who report:
sp. gr. at 15.5°, 0.963; ref. index at 40°, 1.4700; solidificati point -4°C; $[\alpha]_D^{33}$ +2.7°; acid value 1.8; saponification value 184.5; iodine value 91.4; acetyl value 83.5; thiocyanogen valu calculated as % iodine, 89 and unsaponifiable material 0.88 %.

Based on the high sp.gravity, ref. index, optical activit and acetyl value, the occurrence of ricinoleic acid (or an isomer) has been suggested. From the fractional crystallisatio

of the lead salts of the fatty acids (ether and light petroleum and subsequent examination of each fraction an approximate composition of the oil was computed. The lead salt separation gave three fractions in which the saturated, hydroxy and unsaturated acids , respectively were concentrated. From the iodine value, saponification value and acetyl value of each of the fractions, the following composition of the oil was obtained:

oleic 59%, ricinoleic 33%, linoleic 6% and saturated acids 2%.

In view of our experience with Vernonia anthelmintica[1] and Strophanthus seed oils[15], it was thought desirable to re-examine this seed oil and confirm the structure of the hydroxy acid. Preliminary examination showed the occurrence of an epoxy acid, later confirmed as cis-12:13-epoxyoctadec-9-enoic acid and identical with that already discovered in Vernonia anthelmintica seed oil[1,3]; this may be accompanied by a little threo-12:13-dihydroxyoleic acid, but there was no evidence of a monohydroxy acid.

Experimental:

Quantitative Determination of Component Acids:

The oil was extracted with light petroleum (b.p. 40-60°) from a sample of the seeds kindly supplied by Mr. D. W. Grindle. The seeds averaged 0.091 g. in weight and gave 32.7% of a greenish-brown oil which solidified when kept at 0°C. Preliminary examination showed the occurrence of an epoxy acid (61.7% wt., as epoxyoleic acid glyceride) as determined by the method of King[112]. Experiments were first carried out to identify the epoxy acid present and it was found to be identical with that obtained from *Vernonia anthelmintica* seed oil[1]. It is, however, more convenient to describe first the component acid analysis of the oil.

The oil was first treated with acetic acid and then with alcoholic potassium hydroxide solution thereby converting the epoxy acid into the corresponding dihydroxy acid. The mixed hydroxy acids were partitioned between light petroleum (40-60°) and 80% methanol, the acids being first dissolved in the latter. The non-hydroxy acids (petroleum extract) when crystallised from methanol at -20° C gave fraction A (insoluble) and fraction B (soluble). Each fraction was esterified with methanol and sulphuric acid (A) or with methanolic hydrogen chloride (B & C) and B and C were subsequently acetylated. Fractions A and B were fractionally distilled and their composition computed in the usual way. Fraction C was calculated in terms of dihydroxy-

oleic acid, unsaponifiable material and non-hydroxy acids having the same composition as in fractions A and B together; the content of dihydroxyoleic acid was calculated from the saponification equivalent of the esters before and after acetylation.

Dihydroxystearic acid was considered absent since a concentrate of the dihydroxy acid gave only a little dihydroxy-oleic acid when crystallised from ethyl acetate (cf. Riley[105]).

When the mixed acids which had not been treated with aceti acid were partitioned between light petroleum (b.p. 40-60°) and 80% methanol, some dihydroxyoleic acid was isolated. This may have been present as such in the seed oil. The analytical results suggest that 3.4% (mol.) of dihydroxyoleic acid is present in the original seed oil (see page 63).

The results of the analysis are given in the following tables.

Table 8. Characteristics of C. cordofanus Seed Oil:

	Present Work.	Previous Work.[4]
Oil:		
Saponification equivalent	304.9	304.1
Iodine value	91.4	91.4
Free acidity (% oleic acid)	6.8	0.9
Epoxyoleic acid glyceride (% wt.)	61.7	-
$[\alpha]_D^{16.5}$ in acetic acid	+ 3.4°	-
Unsaponifiable material (% Wt.)	1.2	1.0
Mixed Hydroxy Acids[a].		
Saponification equivalent	300.8	293.0[b]
Iodine value	99.1	-
Absorption $E_{1cm.}^{1\%}$ at 234 mμ, (180°/60 min.)	144.8	-

a. Mixed Hydroxy Acids: This refers to the mixed acids prepared after conversion of the epoxy acid to the corresponding dihydroxy acid.

b. These are mixed acids without initial conversion of the epoxy acid to the dihydroxy acid.

Table 9. Partition of Mixed Hydroxy Acids Between 80% Methanol and Light Petroleum (b.p. 40-60°).

To 1 litre of 80% methanol in each of the three separatory funnels (1-3) was added about 50 g. of the mixed hydroxy acids and 500 ml. of the solvent was placed in funnels 4 and 5. Light petroleum (250 ml.) was added to the first funnel and after equilibrium passed to each of the other four funnels in turn. This was followed by other portions of light petroleum (250 ml. each time) until little or no material was extracted from the methanol solutions. The distribution of the material was as follows:

	Methanol Solutions.					Petroleum Extracts.				
No.	1	2	3	4	5	5	4	3	2	1
Wt.g.		103.0		4.1		1.0	1.7	3.7	9.1	30.4
		107.1 (C).				45.9 (A + B).				

Table 10. Low-Temperature Crystallisation of Non-Hydroxy Acids

The non-hydroxy acids (45.9 g.) were crystallised from methanol (460 ml.) at -20° C giving fraction A (insoluble) and fraction B (soluble).

Fraction.		Wt.(g.)	% (Wt.)	Iodine Value.
A.	Petroleum extract, insoluble in methanol at -20°C.	9.0	5.9	7.7
B.	Petroleum extract, soluble in methanol at -20°C.	36.9	24.1	140.6
C.	Methanol extract.	107.1	70.0	91.3

Table 11. **Distillation Data - Fraction A.**

No.	Wt., g.	I.V.	S.E.	C°_{16}	C°_{18}	C°_{20}	$C^{'}_{18}$	N.S.
A1	2.01	0.9	271.7	1.91	0.08	-	0.02	-
A2	3.11	3.0	276.9	1.58	0.46	-	0.07	-
A3	1.92	7.6	293.6	0.30	1.45	-	0.17	-
A4	1.36	6.5	298.2	0.01	1.15	-	0.10	-
A5*	1.48	10.9	373.6	-	0.25	0.91	0.10	0.2
T.	8.78			3.80	3.39	0.91	0.46	0.2
		% Esters.		43.28	38.61	10.36	5.24	2.5
		% Acids.		43.09	38.63	10.41	5.24	2.6
	Increment (5. 9%).			2.54	2.28	0.61	0.31	0.1

* 1.279 g. of this fraction contains 0.194 g. of unsaponifiable material.

Table 12. Distillation Data - Fraction B.

No	Wt., g.	I.V.	S.E.	$C^{\bullet}_{16}$	C°_{18}	C°_{20}	C'_{18}	C''_{18}	C'_{18} diOAc	N.S.
B1	2.84	101.3	289.3	0.83	-	-	0.69	1.32	-	-
B2	2.73	140.7	293.5	0.05	-	-	0.92	1.76	-	-
B3	2.86	143.2	294.7	-	-	-	0.98	1.88	-	-
B4	2.98	144.7	294.7	-	-	-	0.97	2.01	-	-
B5	2.82	144.3	295.2	-	-	-	0.93	1.89	-	-
†B6	2.76	142.9	293.7	-	-	-	0.95	1.81	-	-
B7	3.11	142.5	295.0	-	-	-	1.09	2.02	-	-
B8	2.50	141.5	293.3	-	-	-	0.90	1.60	-	-
B9	2.88	138.1	294.4	-	-	-	1.15	1.73	-	-
B10	1.98	135.2	290.7	-	-	-	0.78	1.18	0.02	-
‡B11	3.27	88.8	245.4	-	-	-	0.50	0.75	1.25	0.77
T.	30.73			0.88	-	-	9.86	17.95	1.27	0.77
% Esters.				2.86	-	-	32.09	58.41	4.13	2.51
% Acids.				2.87	-	-	32.34	58.81	3.33*	2.65
Increment (24.1%).				0.69	-	-	7.79	14.18	0.80*	0.64
Acids in fraction A.				2.54	2.28	0.61	0.31	-	-	0.16
Acids in A + B.				3.23	2.28	0.61	8.10	14.18	0.80*	0.80
% Acids in A + B, excluding non-sap., and dihydroxyoleic.				11.37	8.03	2.15	25.82	49.93	-	-

* Calculated as dihydroxyoleic acid.

‡ B11. 2.443 g. of this fraction contains 0.573 g. of non-sap.

† B6 Acids, iodine value 151.5,
ultra-violet absorption, $E^{1\%}_{1cm}$ (180°/60 min.) at 234mμ 598.0
" " at 268mμ 5.5

Table 13. **Fraction C.**

Saponification equivalent of esters before acetylation = 326.1
(Mean of 325.4, 326.0, 326.5, 326.4)

Saponification equivalent of esters after acetylation = 141.5
(Mean of 141.6, 141.2, 141.6, 141.6)

Unsaponifiable material in esters. = 0.43 %.

Hence, composition of C. (% wt.): Dihydroxyoleic acid = 93.5
Non-hydroxy acids = 6.1
Unsaponifiable = 0.4

The non-hydroxy acids in fraction C are assumed to have the same composition as that of fractions A and B together without dihydroxyoleic acid and unsaponifiable material. Hence the composition of fraction C works out as follows:

	C°_{16}	C°_{18}	C°_{20}	C'_{18}	C''_{18}	Dihydroxy oleic	N.S.
C acids.	0.48	0.34	0.09	1.21	2.13	65.45	0.30
[Increment, 70.0%].							

Table 14. Component Acids of C. cordofanus Seed Oil.

Acid.	A	B	C	Total	Excluding unsaponifiable.		
					%(Wt)	%(Mol)	%(Wt
Palmitic	2.54	0.69	0.48	3.71	3.8	4.4	3.9
Stearic	2.28	-	0.34	2.62	2.6	2.8	2.8
Arachidic	0.61	-	0.09	0.70	0.7	0.7	0.7
Oleic	0.31	7.79	1.21	9.31	9.4	10.0	9.8
Linoleic	-	14.18	2.13	16.31	16.5	17.8	17.1
Dihydroxy-oleic	-	0.80	65.45	66.25	67.0	64.3	3.7
Epoxyoleic	-	-	-	-	-	-	62.0
Unsaponi-fiable	0.16	0.64	0.30	1.10	-	-	-

* The quantity of epoxyoleic acid glyceride determined directly (60.9% mol.) is less than the quantity of dihydroxy-oleic acid (64.3% mol.); the difference is considered to be due to some dihydroxyoleic acid originally present. This final column gives the composition of the original acids on a weight basis.

Identification of Individual Acids:

Palmitic acid, m.p. 62-63.5° was isolated from fraction A1 and confirmed by mixed melting point with an authentic sample.

Stearic acid, m.p. 68.8-69.0° was obtained from fraction A4 and similarly confirmed.

Oleic acid. A concentrate of this was obtained from B3 - B9 as a complex with urea and was oxidised with dilute alkaline potassium permanganate[116] to erythro-9:10-dihydroxystearic acid m.p. 129-129.5°, raised to 129.5-130.5° when mixed with an authentic sample.

Linoleic acid, present in fractions B3-B9 was converted to 9:10:12:13-tetrabromostearic acid by reaction with bromine in petroleum (b.p. 80-100°) at 0°C. The melting point (113.5-114.5°) was unchanged when mixed with an authentic sample.

Epoxyoleic acid. A pure sample of this could not be obtained either by crystallisation of the mixed acids from solvents or by partition between light petroleum (b.p.40-60°) and 80% methanol. The unknown epoxy acid was, however, converted to threo-12:13-dihydroxyoleic acid, m.p. 54° (see page 149) and to erythro-12:13-dihydroxyoleic acid, m.p. 88° (see page 152), identical with those prepared from Vernonia anthelmintica seed oil[1] and must be, therefore, cis-12:13-epoxyoctadec-9-enoic ac

Dihydroxyoleic acid. The small proportion of dihydroxy acid accompanying the large amount of epoxy acid was found to be threo-12:13-dihydroxyoctadec-9-enoic acid, identical with that

prepared from the epoxy acid by hydrolysis. This was proved by the melting point of the acid (54-56°), its p-bromophenacyl ester (72-73°), the corresponding dihydroxystearic acid (95-96°) and its p-bromophenacyl ester (102-105°) all of which remained undepressed when mixed with authentic specimens.

Discussion:

The results indicate that cis-12:13-epoxyoleic acid is the major component acid of the seed oil and that no ricinoleic acid or its isomer is present as previously suggested[4]. When Henry and Grindley[4] examined the oil, it was not recognised that epoxy acids occurred naturally in fats. Since the identity of the oxygenated acid was based merely on the analytical values and not checked by degradative studies, it is not surprising that this unusual acid was overlooked. Unless the component acids present in a fat as computed from analytical data are individually identified, the assumptions made in the calculation cannot be justified and the occurrence of unusual and interesting acids may be overlooked as in the seed oils of Strophanthus[15], Ximenia[11,17] and Vernonia species[1,3].

Most seed oils consists of mixtures of palmitic, oleic, linoleic and linolenic acids. However, the botanical families, Rosaceae, Euphorbiaceae and Cucurbitaceae differ from others in that while many of their seed fats contain the usual acids, several of their species contain some unusual acid like, ricinoleic, licanic, parinaric, elaeostearic, kamlolenic and decadienoic acid. This list must now be extended to include cis-12:13-epoxyoleic acid which also occurs in one species of Compositae (Vernonia anthelmintica)[1,3] and in Malvaceae (Okra seed oil)[5].

Strophanthus hispidus Seed Oil

Introduction:

The genus Strophanthus comprises [118] about 45 species most of which are climbers of tropical forests whilst others are shrubs of the savannah. Approximately 35 species grow in Africa, 10 in Asia and one ot two in Madagascar.

Most of the plants bear beautiful flowers which are distinguished by their ribbon or thread-like prolongations of the corolla lobes. The fruits which sometimes take almost a year to ripen consist of two long carpels which vary considerably in thickness from species to species and contain a number of oil-bearing seeds which are rather flat, spindle-shaped, about 1-2 cm. long and 5 mm. broad. The shell and the endosperm of the seeds are thin and the embryo forms the main part of the seeds. The seeds of the different species all look alike and it is extremely difficult to recognise the species from the examination of the seeds alone. It is, however, easy to recognise the species while the plant is blooming or fruiting.

Natives in many parts of Africa use the seeds of various species for the preparation of highly active arrow poisons. Strophanthus preparations have been used medicinally since 1865 and have been studied considerably on account of the steroid "sarmentogenin". This contains an oxygenated atom at the 11-position and is, therefore, a potential starting point for the partial synthesis of cortisone [119] which has extra-ordinary therapeutic effects on rheumatoid arthritis.

The seed oils do not appear to have been examined in any great detail and only very brief reports are available for som of the seed oils prior to 1952.

Based on a comparision of iodine value with thiocyanogen value two samples of a Strophanthus oil (species unstated) are reported[120] to contain: saturated acids (25.2, 26.6%), oleic acid (44.3, 48.3%), and linoleic acid (30.5, 25.3%).

A Strophanthus seed oil (species not stated) was examined by Kuhn[121] who quotes: acid value 15.2, sap. value 188.8, and iodine value 90.3.

The characteristics of the oils contained in different parts of the seeds of S. Kombé, S. hispidus and S. gratus, hav been described by Tocco and Sanna[122]. The oils in the endosper and cotyledons are straw colored, clear transparent oils with a pungent odour and a nauseating taste. Those extracted from t hulls of the first two species are green in colour whilst that from S. hispidus is white. They all have a rancis odour and a disagreeable taste.

The oil content of Strophanthus sarmentosus[119] seeds vary the "Savannah" type contains 35% whilst the " Forest " type contains only 20% of oil.

Strophanthus sarmentosus, S. hispidus and S. courmonttii seed oils were first analysed in detail by Gunstone[15] who has reported the occurrence of an unusual acid. A concentrate of this acid was obtained by a combination of low-temperature crystallisation and distillation, and despite its high iodine

value, catalytic hydrogenation indicated one double bond. Degradative and other studies showed the acid to be 9-hydroxy-octadec-12-enoic acid. The component acids reported are as follows:

	S.s. (S)	S.s. (F)	S.h.	S.c.
Myristic	0.2	0.2	0.1	0.1
Palmitic	11.9	12.2	11.9	13.4
Stearic	9.2	8.1	7.0	4.5
Arachidic	4.0	3.1	2.0	2.8
Oleic	37.7	43.5	35.5	38.6
Linoleic	29.7	26.4	30.0	30.4
Hydroxyocatdecenoic	7.3	6.5	13.5	10.2

All the above values are in % weight.

S.s. (S) : Strophanthus sarmentosus (Savannah type).

S.s. (F) : Strophanthus sarmentosus (Forest type).

S.h. : Strophanthus hispidus.

S.c. : Strophanthus courmonttii.

The purpose of the present investigation was to see how successfully the method developed for oils containing high proportions of dihydroxy acids (from epoxy oils) could be applied to oils containing low proportions of monohydroxy acid and secondly to try to devise a quicker method of analysing Strophanthus seed oils.

Experimental:

Quantitative Determination of Component Acids:

The oil used was from the same sample as that previously examined by Gunstone[15]. The mixed acids free from unsaponifiabl material were obtained by the usual method and were submitted to the following procedures.

(1). The mixed acids were esterified with methanolic hydrogen chloride and subsequently acetylated. The content of hydroxy-octadecenoic acid was computed from the saponification equivale of the esters, determined in quadruplicate, before and after acetylation. The mixed acids were isomerised with ethylene-glycol-potassium hydroxide and the absorption ($E^{1\%}_{1cm.}$) at 234 mμ measured, whereby the content of linoleic acid was obtained. The balance was the sum of oleic and saturated acids, but oleic acid could not be calculated from the iodine value due to unsatisfactory iodine value in presence of the hydroxy acid. The appropriate data are given in Tables 18, 19 and the results in Table 21.

(2). The mixed acids were partitioned between 80% methanol and light petroleum (b.p. 40-60°), the acids being first dissolved in the latter solvent. The methanol extract (fraction C) was methylated with methanolic hydrogen chloride and subse-quently acetylated. From the saponification equivalent of the esters before and after acetylation, the content of hydroxy-octadecenoic acid in fraction C was calculated. From the iodine

value of the petroleum fraction (fractions A + B) and the absorption ($E^{1\%}_{1cm.}$) at 234 mμ after alkaline isomerisation (180°/60 min.), the content of oleic and linoleic acids was calculated, the iodine value being corrected for the small amou (2.65%) of hydroxyoctadecenoic acid present in this fraction [calculated from the value later found from fraction B alone, procedure (3)]. It was assumed that this small amount of the hydroxy acid did not have adverse effect on the iodine values determined. The balance in this fraction was saturated acids. The non-hydroxy acids in fractions C were assigned the same composition as in fractions A and B together. The appropriate data are given in Tables 16, 17, 18, and 19, and the results in Table 21.

(3). A portion of the non-hydroxy acids (fractions A + B) was crystallised from methanol at -20°C giving fraction A (insolubl and fraction B (soluble). From the iodine value of fraction A, the content of oleic acid in that fraction was calculated, the balance being saturated acids. Fraction B was methylated and subsequently acetylated and the content of hydroxyoctadecenoic acid in the fraction calculated from the saponification equivalent of the esters before and after acetylation. From the iodine value of fraction B and its ultra-violet absorption ($E^{1\%}_{1cm.}$) at 234 mμ after alkali isomerisation at 180°/60 min., the content of oleic and linoleic acid was calculated, after making the necessary ~~correction~~ iodine value correction for the amount of the unsaturated hydroxy acid present. The balance was

saturated acids. Fraction C was computed as hydroxyoctadecenoic acid [obtained from (2)] and non-hydroxy acids with the same composition as in fractions A and B together. The appropriate data are given in Tables 16, 17, 18, 19, and the result in Table 21.

(4). Fraction B was methylated, acetylated and distilled under reduced pressure through an electrically heated and packed column, and the composition of the esters fractions was computed in the usual way. The results for this fraction were combined with those of fraction A [procedure (3)] and fraction C was calculated in terms of hydroxyoctadecenoic acid [from procedure (2)] and non-hydroxy acids with the same composition as in fractions A and B together. The data are recorded in Tables 16, 17, 18, 19, 20 and the results in Table 21.

Complete details of the procedures are given in the follow tables:

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Table 15. Characteristics of Strophanthus hispidus Seed Oil.

Yield of oil (% wt).	30.0
Iodine value.	98.3
Saponification equivalent.	293.7
Free acidity (% oleic acid)	5.3
Refractive index at 17°	1.4655

Table 16. Partition of Mixed Acids Between 80% Methanol and Light Petroleum (b.p. 40-60°).

To 1 litre of light petroleum in each of the three separatory funnels (1-3) were added about 50 g. of the mixed acids and 500 ml. of the same solvent were placed in funnels 4 and 5. 80% Methanol (500 ml.) was then added to the first funnel and after equilibrium passed to each of the other four funnels in turn. This was followed by other similar portions of 80% methanol until little or no material was extracted from the petroleum solutions. The distribution of the material was as follows:

	Petroleum Solutions					Methanol Extracts.						
No.	1	2	3	4	5	7	6	5	4	3	2	1
Wt., g.	119.8			6.2		0.9	1.3	1.9	2.9	4.2	5.7	5.1
	126.0 (A + B)					23.0 (C).						

Table 17. Low-Temperature Crystallisation of Non-Hydroxy Acids

The non-hydroxy acids (101.3 g.) were crystallised from methanol (1013 ml.) at -20° C giving fraction A (insoluble) and fraction B (soluble).

Fraction.		Wt.(g.)	% (Wt).	Iodine Value.
A.	Petroleum extract, insoluble in methanol at -20° C.	31.4	21.2	11.6
B.	Petroleum extract, soluble in methanol at -20° C.	94.6	63.9	126.7
C.	Methanol extract.	22.0	14.9	96.0

Table 18. Iodine Values and Ultra-Violet Absorption after Alkali Isomerisation.

Fraction.	Iodine Value.	$E^{1\%}_{1cm.}$ (234mμ)	Linoleic (% wt.)	Oleic (% wt)	Saturated. (% wt.)
A (acids).	11.6	-	-	13.9	87.1
B (acids).	126.7	384.4	42.4	52.3	1.8
C (acids).	96.0	-	-	-	-
C (esters).	92.2	-	-	-	-
C (acetylated esters).	75.2	-	-	-	-
A + B (acids).	97.3	297.7	32.9	39.6	24.8
Mixed acids.	104.8	269.2*	29.7	55.9	

*Value has been corrected for 1.32% of unsaponifiable material present in this fraction.

Isomerisation was carried out with 7.5 % potassium hydroxide in ethylene glycol at 180°/60 min..

Table 19. Saponification Equivalents.

Fraction.	Saponification Equivalent of Esters.				% Hydroxy-octadecenoic acid in fraction (acids).
	Before acetylation		After acetylation		
Mixed. (esters)	294.9 294.9 295.3 295.4	295.1	262.1 263.6 263.8 263.1	263.2	15.3
B	295.1 295.3 296.2 295.3	295.5	287.6 286.7 287.5 287.7	287.4	3.5
C	306.1 307.3 307.0 306.8	306.8	179.4 180.0 180.3 179.5	179.8	93.9
A + B	-		-		2.65*

* This value was calculated from the hydroxyoctadecenoic acid content of B (acids) and the weights (%) of A and B.

Table 30. Distillation Data - Fraction B.

No.	Wt., g.	I.V.	S.E.	C°_{16}	C°_{18}	C'_{18}	C''_{18}	C'_{18} OAc.	N.S.
B1	2.85	85.1	282.3	0.97	0.03	0.98	0.86	-	-
B2	2.90	125.0	293.2	0.09	0.04	1.47	1.30	-	-
B3	2.56	128.9	293.1						
B4	2.73	128.9	294.5						
B5	2.77	128.0	294.3						
B6	2.75	126.7	294.3						
B7	3.07	126.6	295.0						
† B8	2.72	126.2	294.8	-	0.47	16.30	14.34	-	-
B9	2.92	124.6	295.1						
B10	2.51	124.7	294.2						
B11	3.17	123.3	293.5						
B12	3.15	121.4	294.9						
B13	2.76	119.1	294.5						
B14	2.14	115.3	292.9	-	0.63	1.11	0.98	0.02	-
‡ B15	2.69	91.4	268.7	-	0.03	0.81	0.71	0.84	0.30
T.	41.69			1.06	0.60	20.68	18.19	0.86	0.30
% Esters (ex. non.sap)				2.56	1.45	49.96	43.95	2.08	-
% Acids (ex.non.sap)				2.56	1.45	50.11	44.04	1.84*	-
Increment (63.9 %).				1.64	0.93	32.03	28.15	1.18*	-

† B8 acids: I.V. 130.5 ; $E^{1\%}_{1cm.}$ at 234 mμ (180°/60 min) 417.9

‡ B15: 1.8817 g. of this fraction contains 0.2064 g. unsaponifiable.

* Calculated as hydroxyoctadecenoic acid.

Table 21. Component Acids of Strophanthus hispidus Seed Oil.

Acid.	Procedure (1)	Procedure (2)	Procedure (3)	Procedure (4)	Previous Work.
Saturated.	55.0	21.4	19.8	21.2	21.0
Oleic.		34.1	36.6	35.1	35.5
Linoleic.	29.7	28.3	27.4	28.5	30.0
Hydroxy-octadecenoic.	15.3	16.2	16.2	15.2	13.5

All figures give % weight free of unsaponifiable material.

Discussion:

A slightly higher content of the hydroxyoctadecenoic acid is obtained in the present work than that previously recorded[15]. The lower content previously recorded may be due to partial decomposition of the acetoxyoctadecenoate during the distillatio of the mixed acetylated esters and since the decomposed acetoxyoctadecenoate would be calculated mainly as linoleate this would also account for the higher proportion of linoleic acid reported by Gunstone[15].

The Strophanthus oils may be analysed in a number of ways with varying degrees of accuracy. Complete analysis may be made by our distribution procedure. More approximate results in terms of saturated, oleic, linoleic and hydroxyoctadecenoic acid may be obtained more quickly by abbreviated procedures. Difficulty arises because of unsatisfactory iodine values. In procedure (1), the iodine value is not used and the saturated and oleic acids are expressed together. The saturated acids could be determined by Bertram's oxidation method[123] and the content of oleic acid would then follow by difference. In other procedures most of the hydroxy acid is removed and it is considered that the iodine value of the remaining material, even though it contains a small amount of the hydroxy acid, may be safely used to calculate (with isomerisation data) the proportions of linoleic oleic and saturated acids. This has been done in various ways in procedures (2) to (4).

Ergot Oil.

Introduction:

Ergot is a generic name for fungi Caviceps purpurea Tulasn occurring upon various cereals replacing the grain and particularly originating in the ovary of the rye (Secale cereal This and other closely related species of the fungus occur in some 200 grasses, wild and cultivated. Adam Lonicer (1582) firs described ergot as a disease of rye and refers to its use by midwives. Ergot remained an unofficial remedy (for quickening child-birth) during the 17th. and 18th. centuries but following the publication of " An Account of Pulvis parturiens- a remedy for quickening child-birth" by John Stearus (1808) it came into general use in the U.S.A. and in Europe. The chief commercial varieties of ergot come from Polland, Russia, Spain and Portuga The main value of ergot lies in the alkaloids it contains but it also contains a fatty oil (ca. 30%).

Ergot oil has been described by various workers as being dark in colour with a slight green fluorescence, a rancid taste and dextro-rotatory. Several investigators[124-131] have mentione its high acetyl value and have reported the presence of myristi palmitic, stearic, oleic and a hydroxyoctadecenoic acid among the component acids. The latter was shown by Matthes and Kurscher[136] to be identical with ricinoleic acid, ozonolysis of which affords[127] 1- β-hydroxypelargonic acid and not the

* Thorpe's Dictionary of Applied Chemistry, fourth edition, 1940, vol. 4, page 326.

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d- isomer as previously reported. Baughman and Jamieson found no evidence of a hydroxy acid and the hydroxy acid was not identified by most of the other workers who reported its occurrence. It was, therefore, decided to re-examine the oil.

The Component Acids of Ergot Oil:

Three samples of ergot oil were obtained from (1) Burrough Wellcome and Co., (1/2 gallon); (2) Carneigies (125 g.); (3) Roussel Laboratories Ltd., (9 g.). All were dark in colour (greenish-brown), possessed a strong disagreeable odour and contained insoluble impurities and volatile solvent. The solid impurities and the solvent were removed prior to investigation. As oil (1) was available in sufficient quantity it was examined in detail whereas the other two were examined only in part.

The analytical characteristics of the oils were first determined; these are listed in Table 23 where they are compare with the values reported by previous investigators.

The variation in saponification equivalents of different oils may be due to the different degree of interesterification and to varying amounts of unsaponifiable material. The iodine values of oils (2) and (3) are lower than that of (1) and may be so because of a lower linoleic acid content as shown by lower absorption at 234 mμ after alkaline isomerisation.

Table 22. Characteristics of Ergot Oil.

	B/W (1)	C (2)	R (3)	(4) 124	(5) 133	(6) 134
Oil:						
Ref. index (20°)	1.4748	1.4730	1.4730	-	1.4790	-
Iodine value.	75.7	69.6	71.3	-	71.6	-
Free acid (% Ol.)	8.6	-	-	-	1.3	6.2
Sap. equivalent.	282.4	280.0	322.8	286.0	302.4	301.8
S.E. (after acetylation)	265.2	-	-	-	-	-
Unsaponifiable %.	2.4	-	13.4	-	0.3	1.4
Mixed Acids:						
Iodine value.	74.6*	-	-	-	-	-
$E^{1\%}_{1cm.}$ at 234mμ, (180°/60 min.).	115.5*	87.0	97.2*	-	-	-
Sap. equivalent.	280.1	-	-	288.0	-	291.6
S.E.(after acetylation)	223.4	-	-	-	-	-
Mixed Esters:						
Sap. equivalent.	-	295.1	-	-	-	-
S.E.(after acetylation)	-	226.4	-	-	-	-

* Free of unsaponifiable material.

Preliminary examination confirmed the presence of a hydrox acid and evidence concerning its structure is described later. A quantitative study of the component acids of oil (1) was made by the method already described (page 24). Full experimental details are given on page 89, but the results, together with those of others, are summarised here and discussed:

Table 23. Component Acids of Ergot Oil.

% Wt.	B/W (1)	C (2)	(3)[125]	(4)[128]	(5)[129]	(6)[132]
Myristic	0.9	30.1	28.0	3.0	Nil.	0.3
Palmitic	23.9			25.0	30.3	21.7
Stearic	3.2			2.1	12.1	5.3
Arachidic	0.9			Nil.	Nil.	0.7
Hexadecenoic	3.8	- *	Nil.	Nil.	Nil.	Nil.
Oleic	20.9	20.7	32.4	20.9	23.2	63.2
Linoleic	12.3	9.6	3.6	13.2	Traces	8.8
Ricinoleic	34.1	39.6	36.0	35.8	34.4	Nil.

There is closed agreement between the present results and those reported by Fiero[128] (4), who, however, failed to detect hexadecenoic acid as did the other workers. The content of ricinoleic acid agrees well with previous results of Matthes ar Schutz[125] (3), Fiero[128] (4), Vandermeulen[129] (5), and it is surprising that Baughman and Jamieson[132] (6), found no ricinolei

* Hexadecenoic acid is assumed to be absent.

acid, but a correspondingly higher content of oleic acid. The contents of oleic and linoleic acids seem to vary somewhat but there is better agreement between the saturated acids which consist mainly of palmitic acid with smaller amounts of myrist stearic and arachidic acids. Vandermeulen[129] has reported somewhat higher proportions of palmitic and stearic acids but no linoleic acid.

The hydroxy acid of ergot oil has been reported[130,131] to be interesterified and this has been confirmed in the present work. The hydroxy acid content of the oil was 34% but the saponification equivalent of the oil dropped only to 365.2 fro 282.4 on acetylation (Table 22) , thus showing interesterifica of ricinoleic acid.

The Hydroxy Acid in Ergot Oil.

A concentrate of the hydroxy acid was prepared by partiti of mixed acids between 80% methanol and light petroleum (b.p. 40-60), the acid being concentrated in the former. The positio of the hydroxyl group was determined by a procedure first described by Baruch[135] and subsequently used by Goldsobel[8], Ro et. al.[136], and Riley[25]. The iodine value of the hydroxy acid suggested one ethylenic linkage and oxidation with potassium permanganate in acetic acid[114] determined its position. The optical identity of the hydroxy acid was also established.

(a). Position of The Hydroxyl Group:

The reactions involved in the procedure are given below:

$CH_3[CH_2]_5CH(OH).CH_2CH{:}CH[CH_2]_7COOH.$

↓ Hydrogenation.

$CH_3[CH_2]_5CH(OH)[CH_2]_{10}COOH.$ (I)

↓ CrO_3

$CH_3[CH_2]_5CO.[CH_2]_{10}COOH.$ (II)

↓ NH_2OH

$CH_3[CH_2]_5.\underset{\underset{HON}{\|}}{C}.[CH_2]_{10}COOH.$ (III) + $CH_3[CH_2]_5.\underset{\underset{NOH}{\|}}{C}.[CH_2]_{10}COOH.$ (IV)

↓ H_2SO_4 ↓ H_2SO_4

(V) $CH_3[CH_2]_5CO.NH.[CH_2]_{10}COOH$ + $CH_3[CH_2]_5NH.CO[CH_2]_{10}COOH$ (VI)

↓ ↓

$CH_3[CH_2]_5COOH$ + $NH_2[CH_2]_{10}COOH$ $CH_3[CH_2]_5NH_2$ + $COOH[CH_2]_{10}COOH$

(VII) (VIII) (IX) (X)

The concentrate of the hydroxy acid was reduced catalytically to hydroxystearic acid (I) and then oxidised to the corresponding keto acid (II). The hydroxystearic acid and the ketostearic acid did not depress the melting point of similar compounds prepared from ricinoleic acid. The mixed oximes (III and IV) were prepared and were subjected to a Beckmann rearrangement (with conc. sulphuric acid) and subsequ hydrolysis by boiling with 60% sulphuric acid. Of the four hydrolysis products, three (VII, IX, and X) were isolated and identified as n-heptanoic acid, n-hexylamine and dodecanedioic acid, respectively. This proved the hydroxyl group to be on carbon atom 12.

(b) Position of The Double Bond:

A further portion of the concentrate of the hydroxy acid was oxidised with potassium permanganate in acetic acid [114]. From the products of oxidation, azelaic and heptanoic acids we isolated and identified. Since the hydroxy acid is known to contain only one double bond (from its iodine value 85.5, Table 25) and to carry a hydroxyl group on carbon atom 12, the isolation of these two acids as oxidation products suggests that the hydroxy acid is 12-hydroxyoctadec-9-enoic acid.

(c) Optical Identity of The Hydroxy Acid:

The optical rotation of the concentrate of the hydroxy acid was measured in acetic acid and this showed the acid to b dextro-rotatory.

The hydroxyoctadecenoic acid when oxidised with dilute alkaline potassium permanganate by Lapworth and Mottram's procedure[116] gave two acids, 9:10:12-trihydroxystearic acids which had the same melting points as those prepared from natural ricinoleic acid. Moreover, their optical rotations we of the same magnitude and sign as those prepared from ricinol acid.

Conclusion:

The hydroxy acid in ergot oil is identical with natural ricinoleic acid and is thus (+)-12-hydroxyoctadec-cis-9-enoi acid.

Experimental:

Quantitative Determination of Component Acids:

The mixed acids free of unsaponifiable material were prepared and separated into two fractions by partition betwee 80% methanol and light petroleum (b.p. 40-60°), the acids bein first dissolved in the latter solvent. The non-hydroxy acids (petroleum extract) crystallised from methanol at -20°C gave fraction A (insoluble) and fraction B (soluble). Each fractio was esterified with methanolic hydrogen chloride (B and C) or with methanol - sulphuric acid (A), all the fractions being subsequently acetylated. The composition of fractions A and B was computed in the usual way after fractional distillation. From the saponification equivalent (determined in quadruplica of the methyl esters of (C) before and after acetylation, the content of the hydroxyoctadecenoic acid in (C) was calculated The balance was found to be unsaponifiable material.

The results are reported in the following tables:

Table 24. Partition of Mixed Acids Between 80% Methanol and Light Petroleum (b.p. 40-60°).

To 1 litre of light petroleum in each of the three separatory funnels (1-3) was added about 40 g. of the mixed acids free of unsaponifiable material and 400 ml. of the same solvent were placed in funnels (4) and (5). 80% Methanol (400 ml.) was added to the first funnel and after equilibrium was passed to each of the other four funnels in turn. This was followed by other portions of 80% methanol (400 ml.) until or little or no material was extracted from the petroleum solutio The distribution of the material was as follows:

Petroleum Solutions. No.	(1)	(2)	(3)	(4)	(5)
Wt.g.	79.2 (A + B).				

Methanol Extracts. No.	8	7	6	5	4	3	2	1
Wt.g.	1.8	2.3	2.2	4.0	5.5	6.9	9.2	6.4
	38.3 (C).							

Table 25. Low-Temperature Crystallisation of Non-Hydroxy Aci

The non-hydroxy acids (79.2 g.) were crystallised from methanol (790 ml.) at -20°C to give fraction A (insoluble) an fraction B (soluble).

		Wt.(g.)	% Wt.	Iodine Value.
Fraction. A	Petroleum extract insoluble in methanol at -20°C.	35.9	30.6	9.5
B	Petroleum extract soluble in methanol at -20°C.	43.3	36.9	119.0
C	Methanol extract.	38.3	32.5	85.5

Table 26. **Distillation Data - Fraction A.**

No.	Wt., g.	I.V.	S.E.	C°_{14}	C°_{16}	C°_{18}	C°_{20}	C'_{16}	C'_{18}	N.
A1	2.33	1.3	266.0	0.33	1.97	-	-	0.03	-	-
A2	2.45	2.1	268.7	-	2.40	-	-	0.05	-	-
A3	2.33	1.5	267.6	-	2.39	-	-	0.04	-	-
A4	2.54	1.4	268.9	-	2.50	-	-	0.04	-	-
A5	2.50	1.3	268.7	-	2.47	-	-	0.03	-	-
A6	2.36	0.4	268.6	-	2.35	-	-	0.01	-	-
A7	2.55	1.7	268.4	-	2.50	-	-	-	0.05	-
A8	2.23	10.8	272.2	-	1.95	-	-	-	0.28	-
A9	2.93	54.4	287.5	-	1.00	0.75	-	-	1.18	-
* A10	3.65	21.9	308.5	-	-	1.90	0.76	-	0.93	0.0
T. 25.87				0.33	19.43	2.65	0.76	0.20	2.44	0.0
% Esters.				1.28	75.11	10.24	2.94	0.77	9.43	0.2
% Acids.				1.27	75.02	10.28	2.96	0.77	9.46	0.2
Increment (30.6%)				0.39	22.95	3.15	0.91	0.24	2.89	0.0

* 2.8473 g. of this fraction contains 0.048 g. of unsaponifiab material.

Table 27. Distillation Data - Fraction B.

No.	Wt., g.	I.V.	S.E.	C°_{14}	C°_{16}	C'_{16}	C'_{18}	C''_{18}	C'_{18} OAS.	N.S.
B1	2.73	71.4	264.0	0.44	0.23	2.06	-	-	-	-
B2	2.94	93.0	278.8	-	0.46	1.21	0.70	0.57	-	-
B3	3.11	120.4	291.9	-	0.04	0.09	1.65	1.33	-	-
B4	3.05	124.8	293.0	-	-	-	1.69	1.36	-	-
B5	2.96	124.1	293.0	-	-	-	1.66	1.30	-	-
B6	3.07	123.4	294.0	-	-	-	1.75	1.32	-	-
† B7	3.74	121.7	292.5	-	-	-	2.20	1.54	-	-
B8	2.78	120.9	293.1	-	-	-	1.66	1.12	-	-
B9	3.08	118.0	292.4	-	-	-	1.93	1.13	-	-
B10	2.46	115.6	292.4	-	-	-	1.62	0.84	-	-
B11	2.34	105.4	268.3	-	-	-	2.21	1.15	0.32	-
‡ B12	3.33	80.8	225.2	-	-	-			1.81	0.18
T. 35.57				0.44	0.73	3.36	17.07	11.66	2.13	0.18
% Esters.				1.24	2.05	9.45	47.98	32.78	5.99	0.51
% Acids.				1.24	2.05	9.48	48.35	33.01	5.33*	0.54
Increment (36.9%)				0.46	0.76	3.50	17.83	12.18	1.97*	0.20

† B7 acids: I.V. 127.7 ; $E^{1\%}_{1cm.}$ at 234 mμ (180°/60min) 324.6, at 268 mμ " 5.8

‡ 2.6253 g. of this fraction contains 0.1442 g. of unsaponifiab

* Calculated as hydroxyoctadecenoic acid.

Table 28. Fraction C.

Saponification equivalent of acids before acetylation = 297.6[a]

Saponification equivalent of acids after acetylation = 171.8[b]

Hence % ricinoleic acid = 97.2 (1)

Saponification equivalent of esters before acetylation = 310.3[c]

Saponification equivalent of esters after acetylation = 177.0[d]

Hence % methyl ricinoleate = 99.5 or

% ricinoleic acid in C- acids = 99.4 (2)

Mean of (1) and (2) = 98.3 % ricinoleic acid in fraction C.

Unsaponifiable material: 1.8927 g. of acetylated esters gave 0.0283 g. of unsaponifiable material + 1.576 g. of fatty acids

Therefore, unsaponifiable material in C-acids = 1.7%.

Thus fraction C is : Ricinoleic acid = 98.3 %

Unsaponifiable = 1.7 %.

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a. Mean of 296.6, 298.2, 298.6, 297.0

b. Mean of 171.9, 171.9, 171.7, 171.6

c. Mean of 309.3, 310.9, 309.8, 311.0

d. Mean of 177.5, 176.4, 177.1, 176.9.

Table 29. Component acids of Ergot Oil

Acid.	A	B	C	Total.	Excluding unsaponifiab	
					% (Wt)	% (Mo
Myristic	0.39	0.46	-	0.85	0.9	1.1
Palmitic	22.95	0.76	-	23.71	23.9	26.1
Stearic	3.15	-	-	3.15	3.2	3.1
Arachidic	0.91	-	-	0.91	0.9	0.8
Hexadecenoic	0.24	3.50	-	3.74	3.8	4.1
Oleic	2.69	17.83	-	20.72	20.9	20.6
Linoleic	-	12.18	-	12.18	12.3	12.2
Ricinoleic	-	1.97	31.95	33.92	34.1	32.0
Unsaponifiable	0.07	0.20	0.55	0.82	-	-

Identification of Individual Acids (Other Than The Hydroxy Ac

Palmitic acid, m.p. 63-63.5°, identical with an authentic sampl was isolated from fraction A5.

Stearic acid, m.p. 69-70° was obtained from fraction A9 and confirmed by mixed melting point with an authentic sample.

Hexadec-9-enoic acid. erythro-9:10-Dihydroxypalmitic acid, m. 136-137° was obtained by dilute alkaline potassium permanganate oxidation[118] of B1 acids, and this was identical with an authe specimen.

Oleic acid. A concentrate of this acid was obtained from acids of fractions B8-B10 as a complex with urea. Dilute alkaline potassium permanganate oxidation[116] of the concentrate gave erythro-9:10-dihydroxystearic acid, m.p. 128.5-130° raised to 130-130.5° when mixed with an authentic sample.

Linoleic acid, present in B5 was converted to 9:10:12:13-tetrabromostearic acid, m.p. 113.5-114°, by reaction with bromine in petroleum (b.p. 80-100°). The melting was unchanged when mixed with a pure sample.

Identification of the Hydroxy Acid:

(a). **Position of The Hydroxyl Group:**

Preparation of 12-hydroxystearic acid (I).- The concentrate C (4 g.) in ethanol (55 ml.) was hydrogenated in the presence of palladium charcoal (0.6 g.) (5%). The hydroxystearic acid (3.9 g after crystallisation from ether melted at 78-80°, unchanged whe mixed with 12-hydroxystearic acid from ricinoleic acid, but depressed (71-74°) when mixed with 9-hydroxystearic acid.

Preparation of 12-ketostearic acid (II).- 12-Hydroxystearic ac (4.56 g.) dissolved in acetic acid (44 ml.) was oxidised with a 10 % solution of chromium trioxide in acetic acid (11 ml.) at room temperature. After 30 minutes the solution was diluted with water (300 ml.), decolourised with sulphur dioxide, filter and the solids dried in a desiccator (4.13 g., m.p. 81-82°). One crystallisation from ether - light petroleum (b.p. 40-60°) raise the melting point (82.5-83°) and this was unchanged when mixed with 12-ketostearic acid prepared from ricinoleic acid, but depressed (73-77°) when mixed with 9-ketostearic acid prepared from Strophanthus seed oils.

Preparation of Mixed Oximes (III and IV).- The ketostearic aci (4.6 g.) dissolved in ethanol (73 ml.) was boiled for 2 hours with hydroxylamine hydrochloride (4 g.), sodium acetate (6 g.) and water (18 ml.). A part of the alcohol was then removed and the mixed oximes (4.7 g.) extracted with ether.

Beckmann Rearrangement and Hydrolysis of The Mixed Amides (V, VI

The mixed oximes (4.7 g.) were heated in concentrated sulphuric acid (30 ml.) for 1 hour at 100° C and the solution then diluted with water (37 ml.) to about 60 % sulphuric acid an refluxed for 3 hours. After further dilution (200 ml.) the mixture was steam-distilled and the distillate (750 ml.) gave th monobasic acid fraction (VII) (0.44 g.) on extraction with ether (5 x 200 ml.). The distillation residue when extracted with ethe (5 x 200 ml.) yielded the dibasic acid (X) (2.64 g.). The aqueou residue was made alkaline with 50 % potassium hydroxide solution and steam-distilled. The steam-distillate (1000 ml.) on extracti with ether (5 x 200 ml.) gave the volatile amine (IX) (0.28 g.). An attempt to isolate the amino acid (VIII) from acidified distillation residue by means of an ion exchange resin did not succeed.

Monobasic Acid Fraction (VII).- The p-bromophenacyl ester of thi fraction was prepared and was found to be identical with that of n- heptanoic acid. The melting point (66-68°) was raised (67-70.5° when mixed with the ester of heptanoic acid, but was depressed (62-63°) when mixed with the derivative of hexanoic acid.

Dibasic Acid Fraction (X).- This was extracted with boiling water (15 x 100 ml.) and the aqueous extract after concentration (30 ml., 0°C) gave a crystalline acid, m.p. 127-129° (0.51 g.), identical with dodecanedioic acid. The portion insoluble in boiling water was probably a mixture of unreacted materials.

Volatile Amine Fraction (IX).- This fraction was shown to be n- hexylamine by preparation of its phenyl-iso-thiocyanate derivative, m.p. 75.5-77°, identical with a similar specimen (m.p. 76.5-77.5°) prepared frm commercial n-hexylamine.

(b) Position of The Double Bond:

Oxidation of Unsaturated Hydroxy Acid.- To the hydroxy acid (4 g. of fraction C) dissolved in purified acetic acid (40 ml.) powdered potassium permanganate (16 g.) was added portionwise s that the temperature did not exceed 50° C. After keeping the solution at 45-50° for 3 hours the acetic acid was removed under reduced pressure, The residue was diluted with water (300 ml.) containing some sulphuric acid, decolorised with sulphur dioxid and then steam distilled. The steam distillation residue on extraction with ether (5 x 200 ml.) gave the dibasic acid (2.98 whilst the distillate (1000 ml.) saturated with salt and extrac with ether (5 x 250 ml.) gave the monobasic acid (0.89 g.).

Dibasic Acid: This was extracted with boiling water (8 x 100 m and the extracts after concentration (25 ml.) and cooling (0° C) deposited crystals (1.46 g., m.p. 95-99°). These gave pure azela acid (0.98 g., m.p. 106-107.5°) identical with an authentic sample when crystallised once from ethyl acetate.

Monobasic Acid: When distilled (11 mm. Hg.) this gave two fractions (0.20 and 0.25 g.). Both readily formed p-bromophenac esters and these were identical with each other and with the ester of heptanoic acid (m.p. 69-70°; 69.5-71° with the derivati

of C_γ acid; 63-64° with the derivative of C_β acid).

(c) Optical Identity with Ricinoleic Acid:

(i) Optical Rotation of C-Acids.- The optical rotation of C- Acids (free of unsaponifiable material) was measured in aceti acid and this showed the hydroxy acid to be dextro- rotatory lik ricinoleic acid. The value ($[\alpha]_D^{18.5}$ + 9.3°, 8dcm., acetic acid is slightly higher than those reported by Straus et.al.[137], ($[\alpha]_D^{21}$ + 7.86°, no solvent) and Green and Brown[138] ($[\alpha]_D^{26}$ + 7.15°, acetone). These differences probably reflect the different solvent used and to a lesser extent the varying purity of the samples investigated.

(ii) 9;10;12-Trihydroxystearic Acids.- C-Acids (1.57 g.) were dissolved in water (157 ml.) containing sodium hydroxide (1.57 g and after dilution with ice-cold water (1260 ml.) were oxidised at 10°C with a 1 % potassium permanganate solution (157 ml.). After 5 minutes the solution was decolorised with sulphur dioxid acidified with concentrated hydrochloric acid (47 ml.) and left overnight. The deposited solids were filtered, dried in a desiccator (1.58 g.) and extracted with boiling petroleum (80-100°; 3 x 25 ml.). The insoluble portion (1.26 g.) on extraction with chloroform (3 x 25 ml.) left an inoluble residu (0.76 g.) which after two crystallisations from ethanol melted at 136.5-137.5°. The chloroform soluble portion was recovered

and this after crystallisation from aq. ethanol gave an acid, m.p. 110-111.5° (0.23 g.). Both derivatives were identical with authentic samples prepared from ricinoleic acid. The optical rotations of these two acids in acetic acid were measured.

Acid m.p. 136.5-137.5°: $[\alpha]_D^{18.5} = -10.7°$ (2 dcm. tube).

Acid m.p. 110-111.5°: $[\alpha]_D^{18.5} = -6.7°$ (2 dcm. tube).

Kass and Rudlove[139] report -11.6° and -6.6° for these acids.

Appendix To Part I. Glycol Value.

Introduction

In the experiments on *Cephalocroton cordofanus* and *Vernonia anthelmintica* seed oils, epoxyoleic acid was converted to the corresponding dihydroxyoleic acid and the content of dihydroxy acid determined from the saponification equivalent of its meth ester before and after acetylation. This method is rather tedi and time consuming and a simpler and quicker method would have been most useful.

Since the dihydroxy acid obtained from the epoxy acid is an α- glycol, it was thought possible to determine the content of hydroxyl groups by periodate oxidation. In addition this would differentiate between α- dihydroxy acids and acids containing one or more hydroxyl groups not in adjacent positio

The action of alkali periodates and periodic acid originally noted by Malaprade[140] in 1928 in oxidising quantitatively α-glycols has found numerous applications. The determination of monoglycerides in oils and fats making use of this reagent was first reported by Pohle *et. al.*[141] and later b Handschumaker *et. al.*[142]. Kummerow and Daubert[143] have stated that the periodic acid oxidation method for the determination of monoglycerides is not very specific. We have confirmed this and in particular we have found the method not very suitable for dihydroxy acids containing unsaturated centres. Considerab improvement was, however, achieved when the reagent used was a solution of potassium periodate in aqueous acetic acid. Since

we discontinued our experiments, Parez et. al.[144], have quoted abnormally high values for the monoglyceride content of variou fish liver oils as determined by periodic acid oxidation, and have suggested that these fictitious results are probably due to the reaction with double bonds present.

Malaprade's reaction may be represented as

$$R'CHOH.CHOH.R'' + HIO_4 = R'CHO + R''CHO + HIO_3 + H_2O$$

The excess reagent and the iodic acid formed are reduced by addition of potassium iodide as follows:

$$HIO_4 + 7KI + 7CH_3COOH = 7CH_3COOK + 4I_2 + 4H_2O$$

$$HIO_3 + 5KI + 5CH_3COOH = 5CH_3COOK + 3I_2 + 3H_2O$$

The amount of periodate reagent consumed, which is determined by titration of liberated iodine with standard sodium thiosulphate solution gives the content of the glycol present.

The reaction between periodic acid and glycol requires 1/4 the total oxidising power of the periodic acid as measured by an iodometric titration. Thus the maximum difference betwee the titration of the blank and that of the sample will be only 1/4 that of the blank. Since an excess of reagent is normally used for quantitative oxidation of the glycol, the titration o the sample will be more than 75 % that of the blank. This reduces the accuracy of the determination. In a later publication, Kruty and others[145] claim to have eliminated this difficulty. They dissolve the periodic acid in a neutral solve

such as 95 % methanol, instead of acetic acid . A known volume of this reagent is added to the glycol dissolved in chloroform and when the oxidation is complete, the reaction mixture is neutralised with sodium bicarbonate. Excess period is reduced to the iodate with liberation of iodine by additio of potassium iodide but no further reduction of the iodate occours in the neutral solution. The liberated iodine is then determined by titration with standard arsenite solution as titration of iodine with thiosulphate in neutral solution lea to erroneous results.

$$H_5IO_6 + 2KI = HIO_3 + 2KOH + I_2 + H_2O$$

$$I_2 + 1/2\ As_2O_3 + H_2O = 1/2\ As_2O_5 + 2HI.$$

Thus maximum difference between the blank and sample titratio can be obtained.

Three different types of periodate solutions were used o different acids, esters and oils and the results are given in Table 30. Reagent A was a solution of potassium periodate in acetic acid containing sulphuric acid. B was a solution of periodic acid in aqueous acetic acid, whereas C was a solutio of potassium periodate in aqueous acetic acid.

Table 30

	Reagent		
	A	B	C
Myristic acid.	0.1	-	0.4
Palmitic acid.	0.1	-	0.3
Stearic acid.	0.4	-	0.8
Oleic acid.	12-16	3.8	1.5-2.7
Methyl linoleate.	19-28	3.9	1.9-3.8
Castor oil mixed acids.	10	-	1.9
Methyl 12-hydroxystearate.	1.5	-	1.5
Groundnut oil.	2.9	-	Nil.
Cottonseed oil.	1.0	-	Nil.
Olive oil.	0.8	-	Nil.
Vernonia anthelmintica seed oil.	77	17	7.4
Cephalocroton cordofanus seed oil	68	13	4.4
9:10-Dihydroxystearic acid, 95°	103	-	101
9:10-Dihydroxystearic acid, 132°	106	-	104
12:13-Dihydroxystearic acid, 95°	102	-	97
12:13-Dihydroxyoleic acid, 54°	162	111	102

Thus best results were obtained by the use of a solution of potassium periodate in aqueous acetic acid and details of the method are given below.

Method: The reagent was prepared by dissolving 1.4 g. of potassium periodate in 200 ml. of water and diluting to 1 litr with glacial acetic acid; stronger solutions could not be prepared because of the low solubility of potassium periodate. The reagent was allowed to stand for a day or two and was filtered immediately prior to its use. The sample, the weight of which was such that iodine liberated after oxidation was no less than 80 % of that liberated in the blank, was dissolved (with warming if necessary) in 10 ml. of a mixture of acetic acid and chloroform (2:1) contained in a glass-stoppered flask 100 ml. of the reagent were added to the solution at room temperature followed after 1/2 hour by 15 ml. of 10% potassium iodide solution and 40 ml. of water. The liberated iodine was titrated with 0.1 N solution of sodium thiosulphate using star as an indicator. A blank was run at the same time. The content of glycol was calculated from:

$$\% \text{ Glycol} = \frac{M.(V_b - V_s).N}{20\ W}$$

where, M= mol.weight of the glycol, N = normality of thiosulp solution, W = weight of the sample, V_b and V_s are the ml. of thiosulphate solution required for the blank and the sample respectively.

Discussion:

The results indicate that the periodate oxidation method is not entirely suitable for the determination of the content of dihydroxy fatty acids containing hydroxyl groups on adjacen carbon atoms. Erroneous results are obtained with unsaturated compounds (confirming Kummerow and Daubert[143], Parez *et. al*[144] although there is considerable improvement when the reagent us is a solution of potassium periodate in aqueous acetic acid. Even than the accuracy of the method is not greater than ± 1. 2.0 % and the reproducibility of the determinations is poor (cf. Hartman[146]). The variation in results is greater with aci than with simple alkyl esters or triglycerides and the method cannot be used in the presence of epoxy compounds.

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Part II. The Preparation of Eight 9:10:12:13-Tetrahydroxystearic Acids.

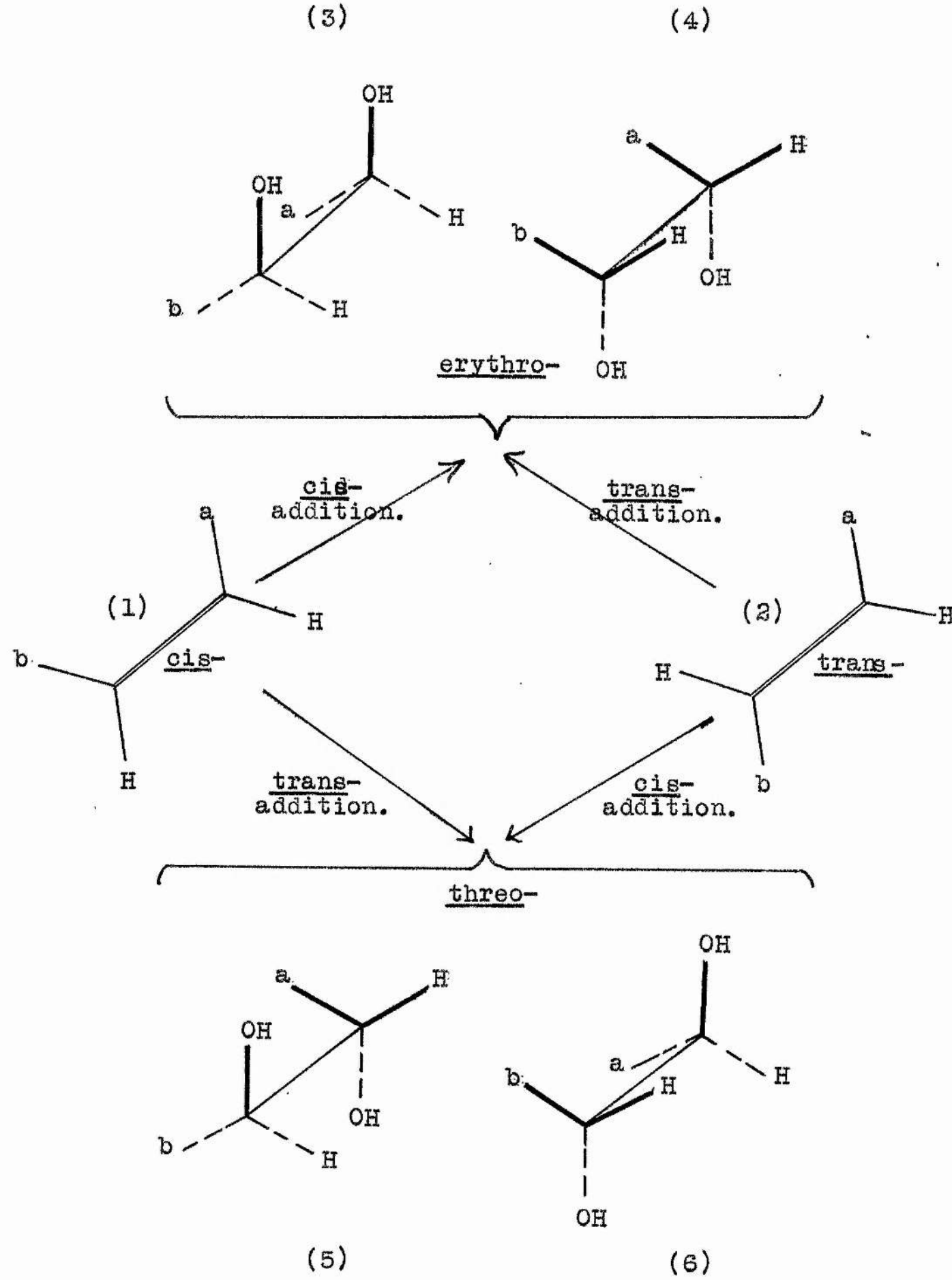
Discussion.

Introduction

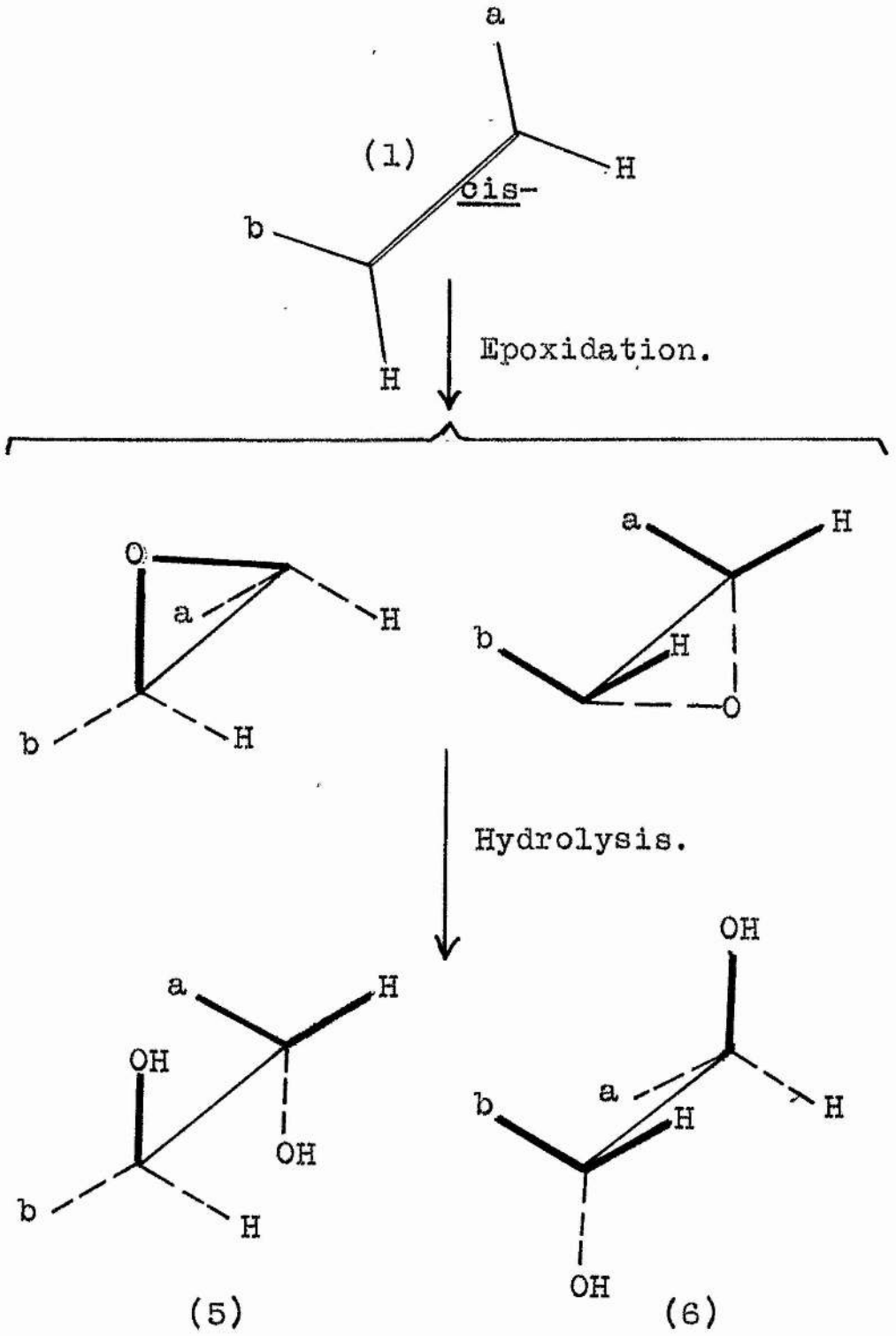
Octadec-9-enoic acid exists in two isomeric forms, the cis- form commonly known as oleic acid and the trans- form called elaidic acid. Oxidation with dilute alkaline potassium permanganate or with peracids like performic, peracetic, etc., gives 9:10-dihydroxystearic acid and since this contains 2 asymmetric carbon atoms it can exist in 4 active and 2 racemic forms.

The relation between oleic and elaidic acids, the two racer 9:10-dihydroxystearic acids derived from them, and various intermediates in their preparation has now been made clear by Swern[1], who has rationalised the stereochemistry of the processe involved and it is now accepted that dilute alkaline permanganat oxidation of the double bond involves cis- addition of hydroxyl groups and that reaction with peracids or hydroxylation proceeding through the halogenohydrins is equivalent to trans- addition of the hydroxyl groups.

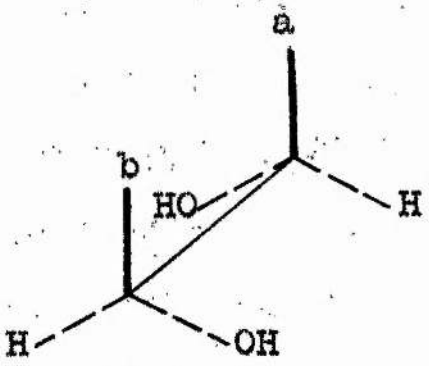
Oleic and elaidic acids may be represented by (1) and (2) respectively. Enantiomorphic dihydroxystearic acids represented by (3) and (4) are then obtained by cis- addition of hydroxyl groups to (1) and also by trans- addition to (2). trans- Additio of hydroxyl groups to (1) will result in the isomers (5) and (6) which are also given by cis- addition to (2) (see page 132). Epoxidation is a cis- addition but since subsequent hydrolysis i accompanied with inversion the overall result is trans- addition


(3)
(4)
OH
OH
a
H
b
H
a
H
b
H
OH
OH
erythro-
cis-
addition.
trans-
addition.
a
H
(1)
b
cis-
H
(2)
a
H
H
trans-
b
trans-
addition.
cis-
addition.
threo-
a
H
OH
OH
b
H
OH
H
a
b
H
OH
(5)
(6)

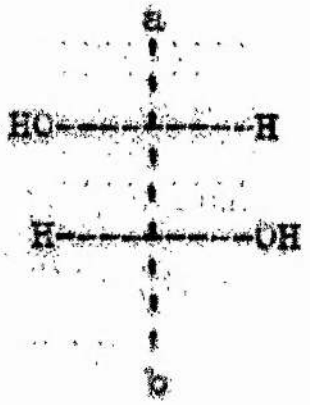
thus the cis- olefin (1) gives the dihydroxystearic acids (5) and (6) as represented below:



Isomer (5) can be rewritten as (7) and its Fischer projection as (10).



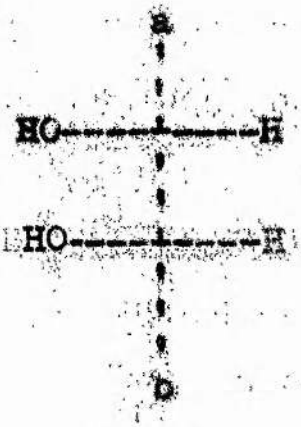
(7)



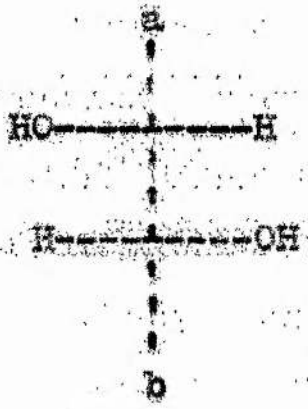
(10)

Thus (3), (4), (5), and (6) can now be represented by (8), (9), (10), and (11) respectively.

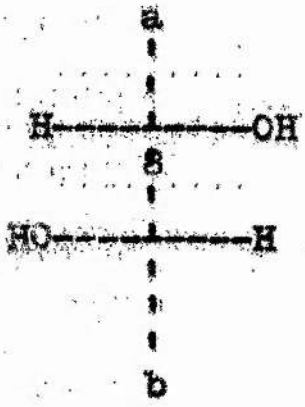
(8)



(9)



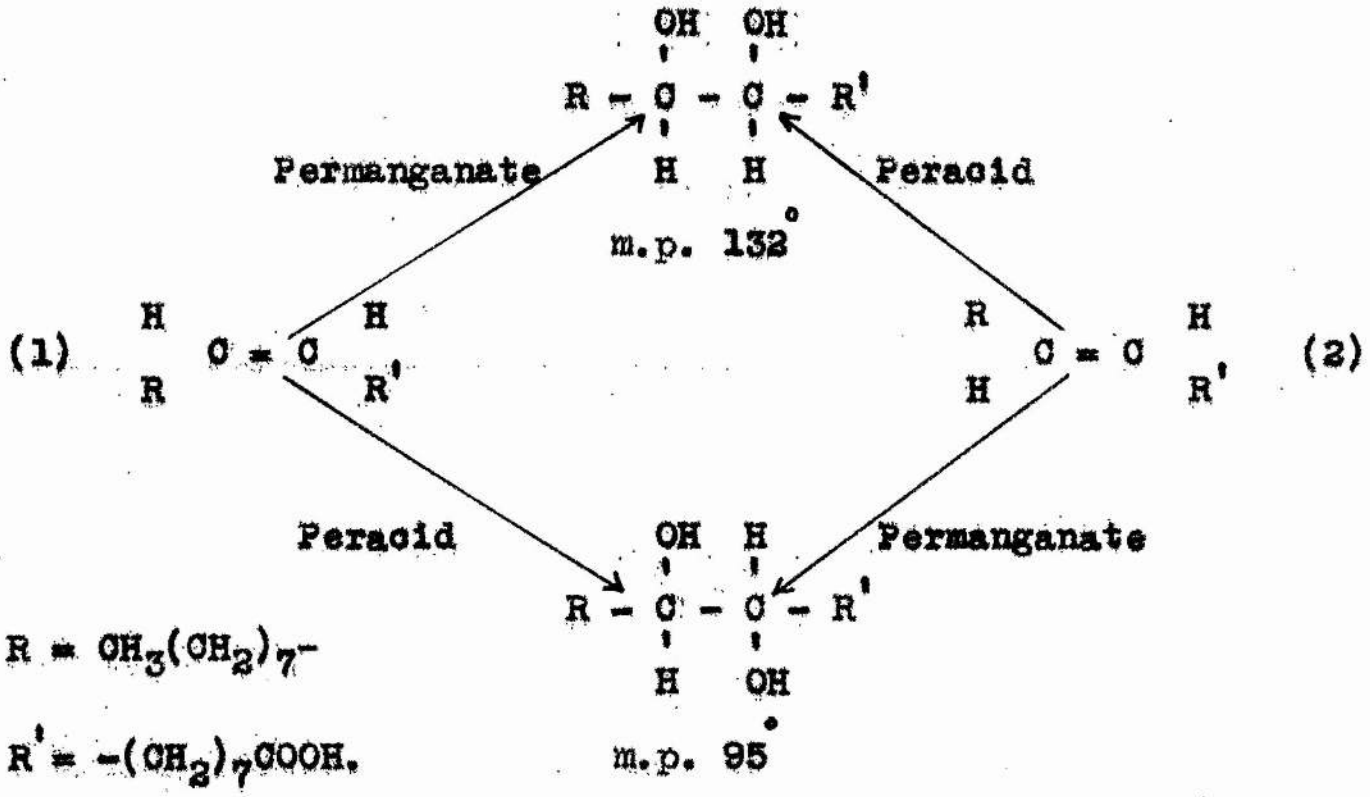
(10)



(11)

Since a and b are different these four (8 - 11) represent the four optical isomers of 9:10-dihydroxystearic acid. Equal parts of (8) and (9) will form one racemate and a second racemate will be given by equal amounts of (10) and (11). In fa it is found that two racemic 9:10-dihydroxystearic acids, m.p.

132° and 95° are formed respectively by the dilute alkaline potassium permanganate oxidation and the performic acid oxidation of oleic acid (1). On the other hand the alkaline permanganate oxidation and the performic acid oxidation of elaidic acid respectively give the 9:10-dihydroxystearic acid, m.p. 95° and 132°.



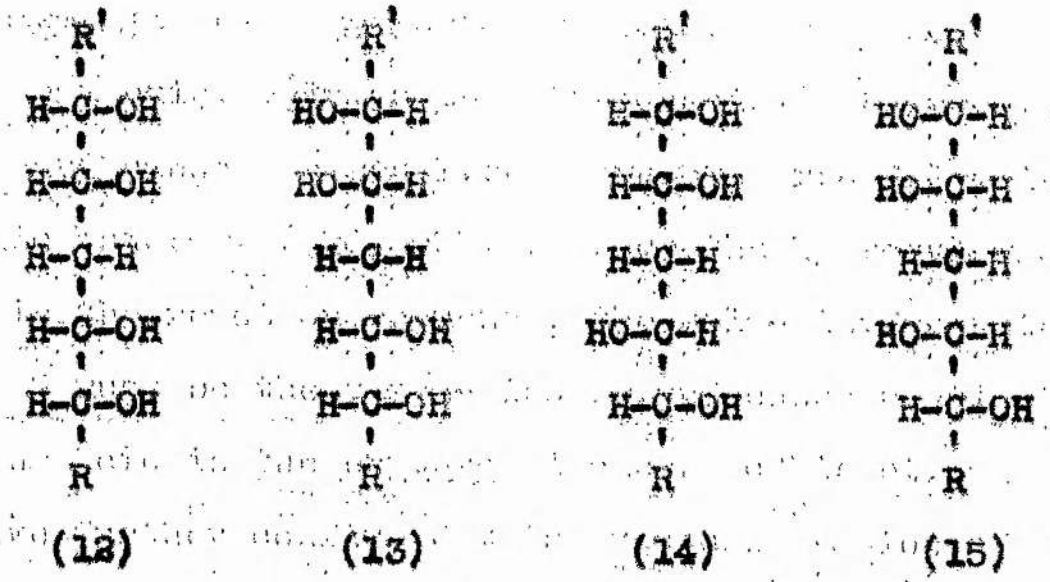
The question now is which of these two racemates has the threo- configuration and which has the erythro- configuration. The answer has been provided from the knowledge of organic reactions[1], X-ray crystallography[1a], urea addition complexes[1b], oxidation with lead tetra-acetate[1c], oxidation with potassium periodate[1d], etc., and it is found that the lower melting acid has the threo- configuration whereas the higher melting has th

erythro- structure. Very conclusive evidence has only recently been provided by Gensler and Schlein[1e], who starting with racemic- 9:10-dihydroxyoctadecanedioic acid, m.p. 131-132°, which can exist only as the threo acid (the erythro- acid is meso- and cannot be resolved) have prepared 9:10-dihydroxystearic acid, m.p. 94.5-95.0°. Since the hydroxyl groups take no part in the reaction sequence, the 9:10-dihydroxystearic acid, m.p. 95° must be the threo- isomer and consequently the higher melting acid is the erythro- isomer. McGhie et. al.[1f], claim to have further confirmed this by consideration of the observed rotation of active 9:10-dihydroxystearic acids.

The terms cis- and trans- used by several investigators to describe the resulting glycols are both confusing and incorrect when applied to open-chain compounds and should be replaced by the terms threo- and erythro-. By definition, threo- compounds result by trans- addition (performic acid oxidation[1]) to a cis- double bond or by cis- addition (dilute alkaline permanganate oxidation[1]) to a trans- double bond, whilst the erythro- isomers are the products of cis- addition to a cis- double bond or trans- addition to a trans- double bond. These rules can now be applied to dienoic acids and the tetrahydroxystearic acids derived from them.

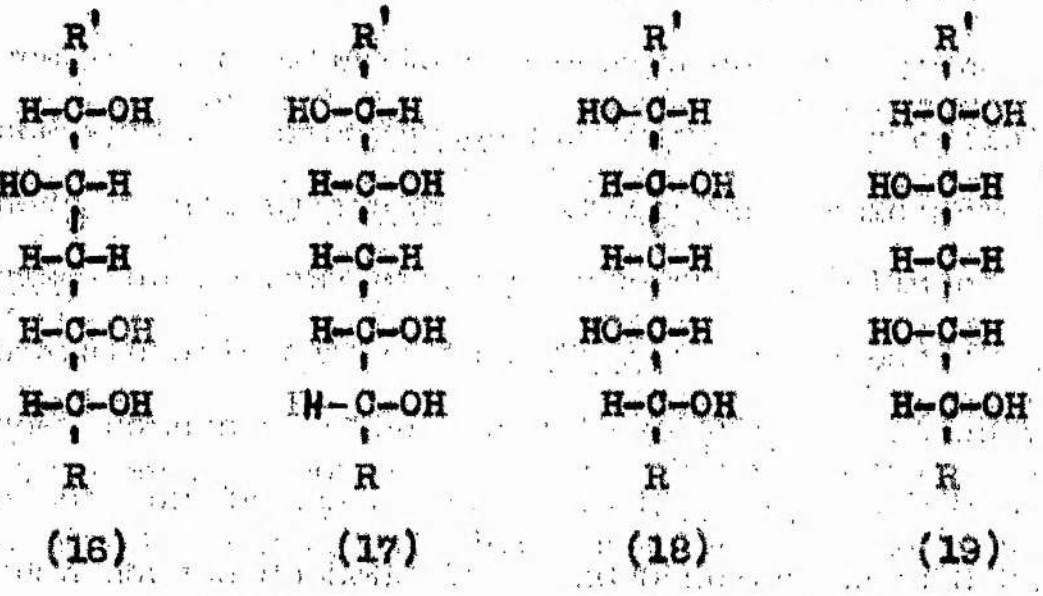
9:10:12:13-Tetrahydroxystearic acid contains four asymmetric centres and can therefore exist in sixteen optically active and eight racemic forms. Eight stereoisomers are shown on page 127 (12 - 19) the other eight being represented by their

enantiomorphs. These are obviously grouped in four pairs.



(12) (13) (14) (15)

erythro-9:10--erythro-12:13- erythro-9:10--threo-12:13-



(16) (17) (18) (19)

threo-9:10--erythro-12:13- threo-9:10--threo-12:13-

$R' = .[CH_2]_7COOH$ $R = CH_3.[CH_2]_4.$

It is now accepted that linoleic acid present in seed oils

is entirely the cis- cis- isomer[2] and that linelaidic acid is the trans- trans- isomer[2]. Oxidation of the double bonds with either dilute alkaline potassium permanganate or performic aci each results in a mixture of two racemates, separated with difficulty and frequently giving eutectic mixtures. It follows that cis- oxidation (dilute alkaline permanganate[1]) of linolei acid affords the two dierythro- isomers which should also result from trans- oxidation (performic acid[1]) of linelaidic acid; similarly the two dithreo- forms are produced from linoleic acid by trans- addition or from linelaidic acid by cis- addition. However, the mixed threo-erythro- isomers (14-1 cannot be produced directly from linoleic or linelaidic acids. In Table 1 the results obtained by several investigators are listed, the configuration of the products being determined on the basis of present views. The general agreement is immediat obvious.

Reference to the mixed threo-erythro- isomers is confine to reports by Kass and Burr[10] and by McKay and Bader[13] (see also page 144). These last authors claim to have prepared all the eight racemic forms starting with linoleic acid. Linoleic acid was brominated with slightly more than one mole of bromine at -15°C. conditions under which the 12:13- double bond was preferentially brominated. The 9:10- double bond was then oxidised either with dilute alkaline permanganate or wit per-acid followed subsequently by debromination (Zn-methanol)

TABLE 1. *Di*-erythro- *and di*-threo-*tetrahydroxystearic acids.*

Date	Investigator	Reactant C_{18} acid	Reagent	M.p.s of di-*erythro*-acids		M.p.s of di-*threo*-acids	
1887	Hazura [3]	Linoleic *	$KMnO_4$	173°			
1909	Rollett [4]	,,	,,	171—173			
1912	Meyer and Beer [5]	,,	,,	173	162—163°		
1922	Nicolet and Cox [6]	,,	,,	170	153		
	,, ,,	,,	Hydrol. of halogeno-hydrin	—	—	144°	135°
1932	Smith and Chibnall [7]	,,	$KMnO_4$	172	159—160	—	—
1935	Green and Hilditch [8]	,,	,,	173	155	—	—
	,, ,,	,,	$AcOH–H_2O_2$	—	—	146	126
1937	Birosel [9]	,,	$KMnO_4$	174	164	—	—
1939	Kass and Burr [10]	Linelaidic	,,	—	—	146	122
1939	Riemenschneider *et al.* [11]	Linoleic	,,	174	163·5	—	—
1939	Hilditch and Jasperson [12]	,,	,,	173	157	—	—
	,, ,,	Linelaidic	,,	—	—	144	135
1948	McKay and Bader [13]	*erythro*-9 : 10-Dihydroxy-*cis*-12-en	,,	174	164	—	—
	,, ,,	*threo*-9 : 10-Diacetoxy-*cis*-12-en	$H·CO_2H–H_2O_2$	—	—	148	126
1954	McKay *et al.*[14]	Linoleic acid	,,	—	—	148	126
1956	Present work †	*erythro*-12 : 13-Dihydroxyoleic	$KMnO_4$	177	156	—	—
		threo-12 : 13-Diacetoxyoleic	$H·CO_2H–H_2O_2$	—	—	148	122

* The linoleic acid is either debrominated tetrabromostearic acid or a concentrate of oleic and linoleic acid prepared from a seed oil containing high proportions of the latter.

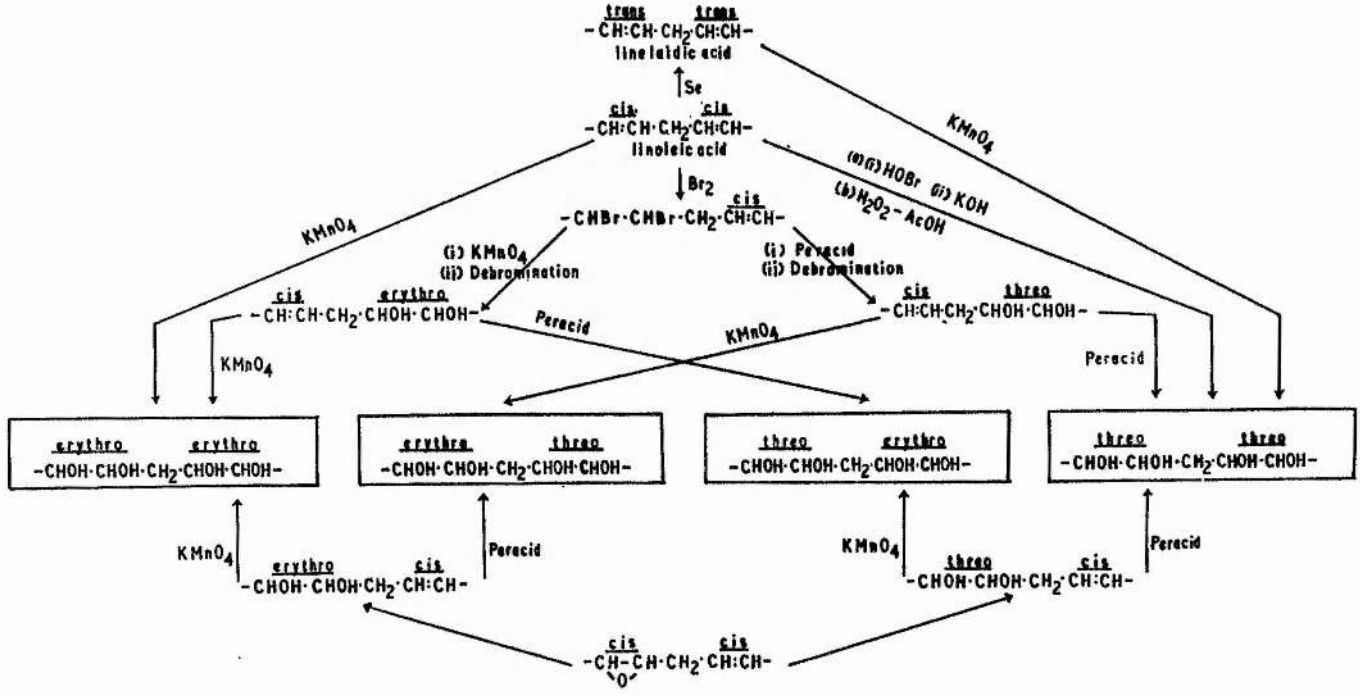
† The products obtained here are optically active (see text) whilst all the others are racemates.

to give erythro-9:10-dihydroxyoctadec-cis-12-enoic acid and threo-9:10-dihydroxyoctadec-cis-12-enoic acid, respectively. The oxidation of the former (after acetylation) with per-acid and the latter with dilute alkaline permanganate gave the diastereoisomers having erythro-9:10-threo-12:13- and threo-9:10-erythro-12:13- configurations (see Figure 1, page 131).

Like most investigators, Mckay and Bader[13] used linoleic acid prepared by debromination of tetrabromostearic acid. α- Linoleic acid so obtained is known[13a] to be contaminated with small quantities of conjugated isomers and with greater proportions of geometrical isomers of linoleic acid. These afford isomeric tetrahydroxystearic acids thereby increasing the difficulty of isolating pure products. Even with pure linoleic and linelaidic acids, the oxidation of each results in a mixture of two racemates which are separated with difficulty and frequently form eutectic mixtures. In view of these difficulties it was considered worthwhile to check these results by an alternative procedure suggested by the availability of 12:13-cis-epoxyoleic acid[15]. Eight 9:10:12:13-tetrahydroxystearic acids have been prepared but these are considered to be optically active for reasons described later

Figure (1)

The 9:10:12:13-Tetrahydroxystearic Acids



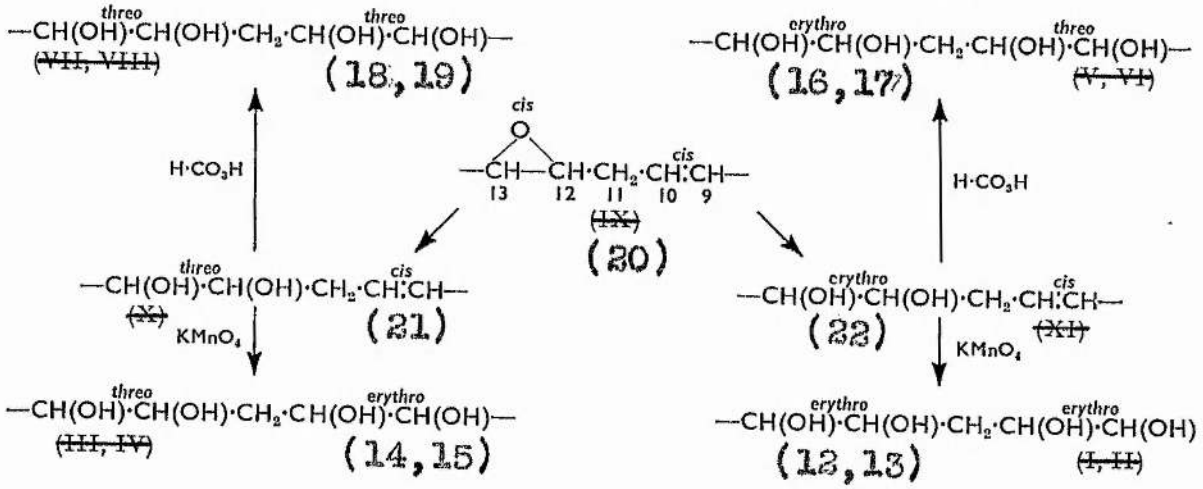
Melting Points

Earlier investigators	174	164 (155)			(157, 127)?			146	122, 126, 135.
McKay and Bader	174	164	164	142	164	126		148	126
Bharucha and Gunstone	177	156	157	130	165	112		148	122

Preparation of threo- and erythro- 12:13-Dihydroxyoctadec--cis-9-enoic Acids.

In part I of this thesis it has been reported that 12:13-cis-epoxyoctadec-cis-9-enoic acid (20) occurs in quantit among the component acids of Vernonia anthelmintica[15] seed oil (74%) and Cephalocroton cordofanus seed oil (66%). Because of the reactive nature of the epoxide ring it is possible to convert it to the threo- and erythro- glycol depending on the geometrical configuration of the epoxide and on the stereochemical nature of the reactions. The threo- and erythro- 12:13-dihydroxyoleic acids (21 & 22) so obtained could then be further oxidised to give 9:10:12:13-tetrahydroxystearic acids as illustrated in Figure (ii).

Figure (ii).

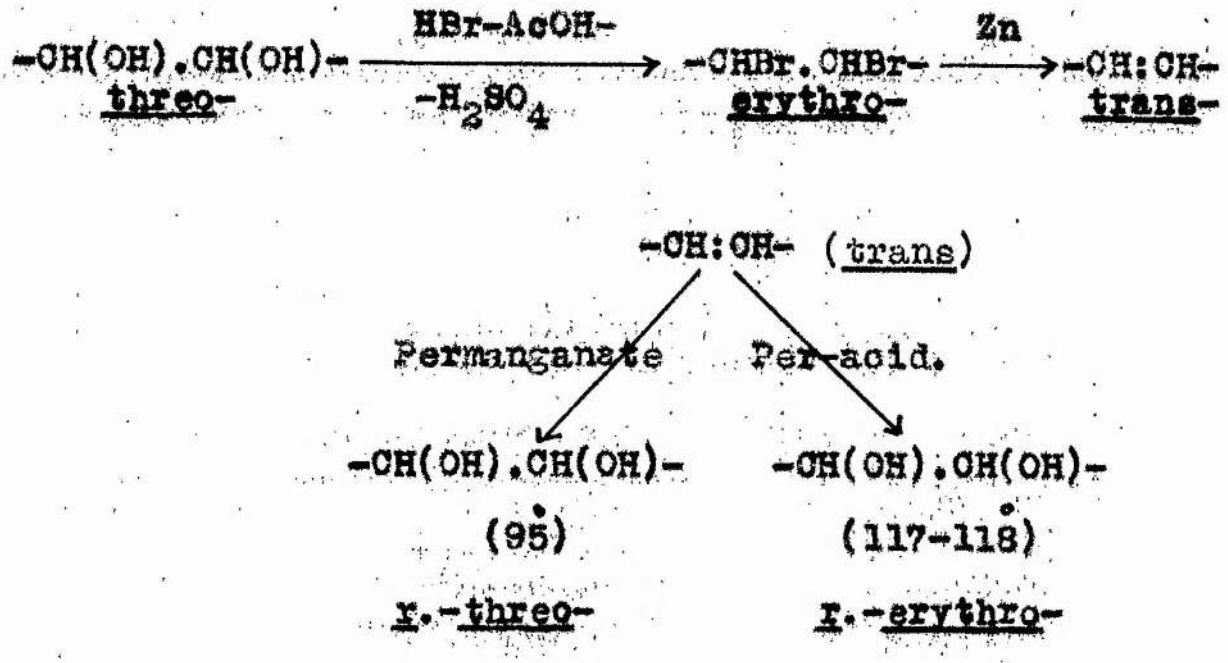


threo-12:13-Dihydroxyoleic Acid (21):

A 12:13-dihydroxyoleic acid, m.p. 54° was obtained on treating the seed oils with glacial acetic acid followed by alkaline hydrolysis. The dihydroxyoleic acid so obtained was considered to be threo-12:13-dihydroxyoleic acid since hydrogenation gave a saturated acid (m.p. 95-96°) of similar m.p. (96.5-97°)[17] to that of the known racemic threo-12:13-dihydroxystearic acid and very different from that of the racemic erythro- isomer (m.p. 119-120°)[17]. The identity of this acid on the basis of melting point cannot, however, be taken as adequate as both the dihydroxyoleic and the dihydroxystearic acids were found to be optically active. An independent proof of the configuration of the hydroxyl groups was therefore necessary and achieved by the partial synthesis of octadec-trans-12-enoic acid.

The 12:13-dihydroxystearic acid (m.p. 95-96°) when brominated by Bowman's procedure[18] and subsequently debrominated gave octadec-trans-12-enoic acid. The identity of this acid is quite certain since the melting point of this acid and of the two racemic- dihydroxystearic acids obtained from it agree with those expected of the trans- acid. Since bromination reaction occurs with an inversion and debromination is a trans-elimination, the dihydroxy compound must be the threo- form. This also provides proof that the epoxide has the cis-configuration since ring opening occurs with an inversion[1] (see Figure iii).

Figure (iii)



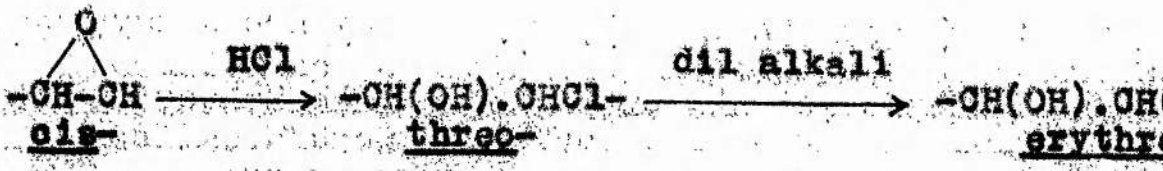
erythro-12:13-Dihydroxyoleic Acid (23):

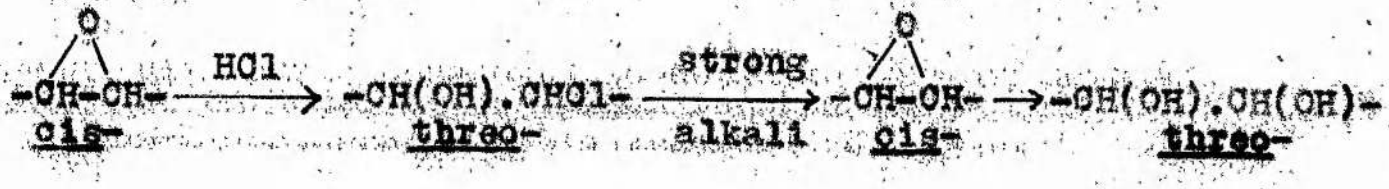
The preparation of this from the cis-epoxide requires a reaction sequence involving no inversion or an even number of inversions. After some unsuccessful attempts (A-C) this was achieved by adaption of some work of Winstein and Buckles[19] (D).

A.

Reaction of an epoxide with hydrogen halides in ether results in the formation of halogenohydrins with inversion[1] which may then be converted to glycols by hydrolysis. With strong alkalies it is reported[20] that an intermediate epoxide is formed which is further hydrolysed to glycol, both reactions being accompanied by inversion. With dilute alkali no intermedi

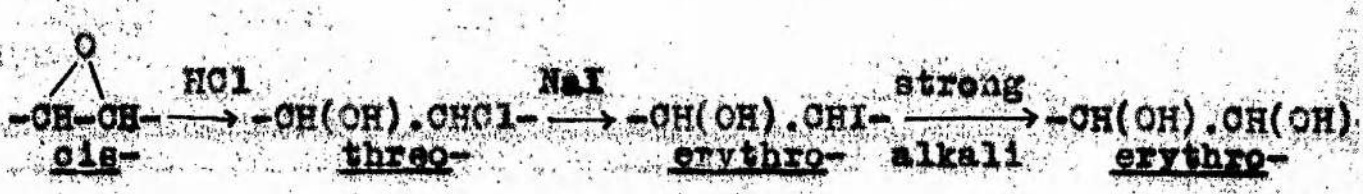
epoxide results and the halogenohydrin is hydrolysed giving the *erythro*- glycol [21].





When *V. anthelmintica* seed oil was treated with hydrogen chloride in ether and subsequently hydrolysed with dilute aqueous potassium hydroxide (0.25N), a small quantity of *threo*-12:13-dihydroxyoleic acid was obtained rather than the expected *erythro*- acid. Replacement of hydrogen chloride by hydrogen bromide gave mixed acids from which no pure dihydroxy acid could be isolated, similar results being obtained when the aqueous alkali was replaced with alcoholic alkali.

B.



The oil after treatment with hydrogen chloride in ether as in **A** was refluxed with sodium iodide in alcohol with the hope of replacing chlorine with iodine with inversion to give *erythro*- iodohydrin. Subsequent hydrolysis with strong alkali would then give the *erythro*- glycol. However, *threo*- dihydroxyoleic acid wa

obtained in small quantity, similar results being obtained when the starting material was treated with hydrogen bromide instead of the chloride.

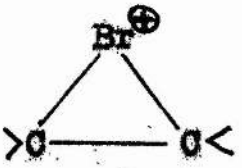
C.

$$\underset{\underline{\text{threo}}}{\text{-CH(OH).CH(OH)-}} \xrightarrow{Ac_2O} \underset{\underline{\text{threo}}}{\text{-CH(OAc).CH(OAc)-}} \xrightarrow{HCl} \underset{\underline{\text{erythro}}}{\text{-CH(OH).CHCl-}} \xrightarrow{\text{strong alkali}} \underset{\underline{\text{erythro}}}{\text{-CH(OH).CH(OH)-}}$$

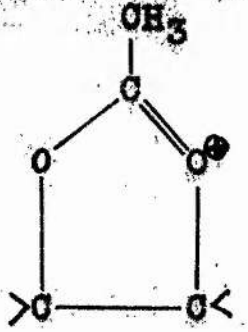
threo-3-Chloro-2-butanol was obtained by Lucas and Gould by reaction of erythro-2:3-diacetoxybutane with concentrated hydrochloric acid in presence of a trace of sulphuric acid. An attempt was accordingly made to prepare erythro-12:13-dihydroxyoleic acid from threo-dihydroxyoleic acid as outlined above, but no pure acid could be isolated from the reaction products.

D. erythro-12:13-Dihydroxyoleic acid (22) was prepared by a reaction sequence based on the work of Winstein and Buckles[19] These workers in the course of investigations of neighbouring group participation in replacement reactions showed that vicinal acetoxybromobutanes and dibromobutanes (also cyclohexane derivatives) react with silver acetate in dry acetic acid to give diacetates with retention of configuration, but in presence of water in the solvent the steric result is shifted towards inversion and with one equivalent of water complete

inversion occurs. This effect has been explained by the participation of the neighbouring group (Br, OAc) in the replacement process. Removal of the halide ion by silver ion results in the formation of intermediates (23) or (24) with inversion.



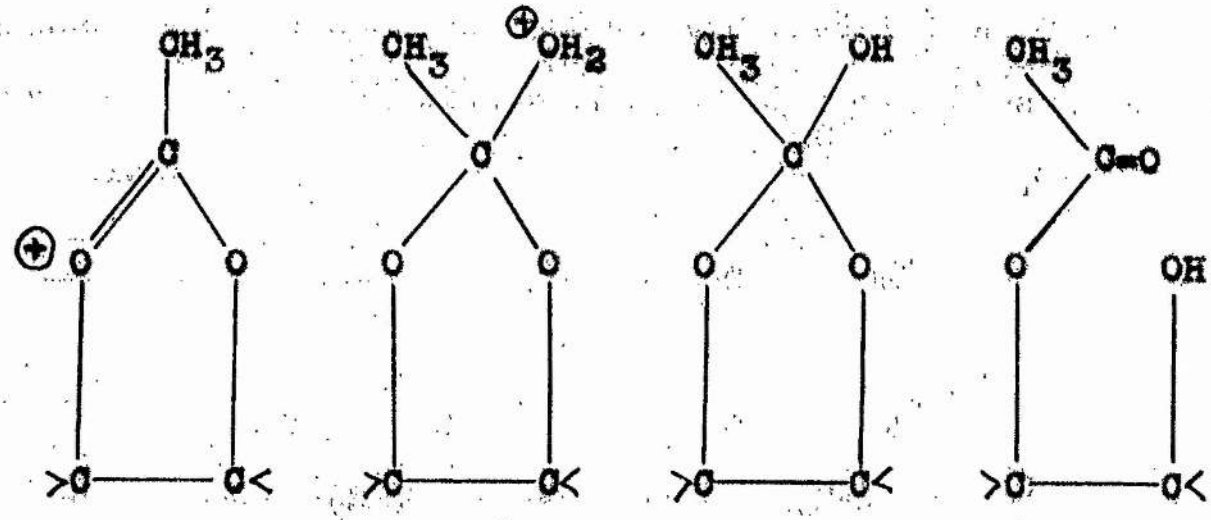
(23)



(24)

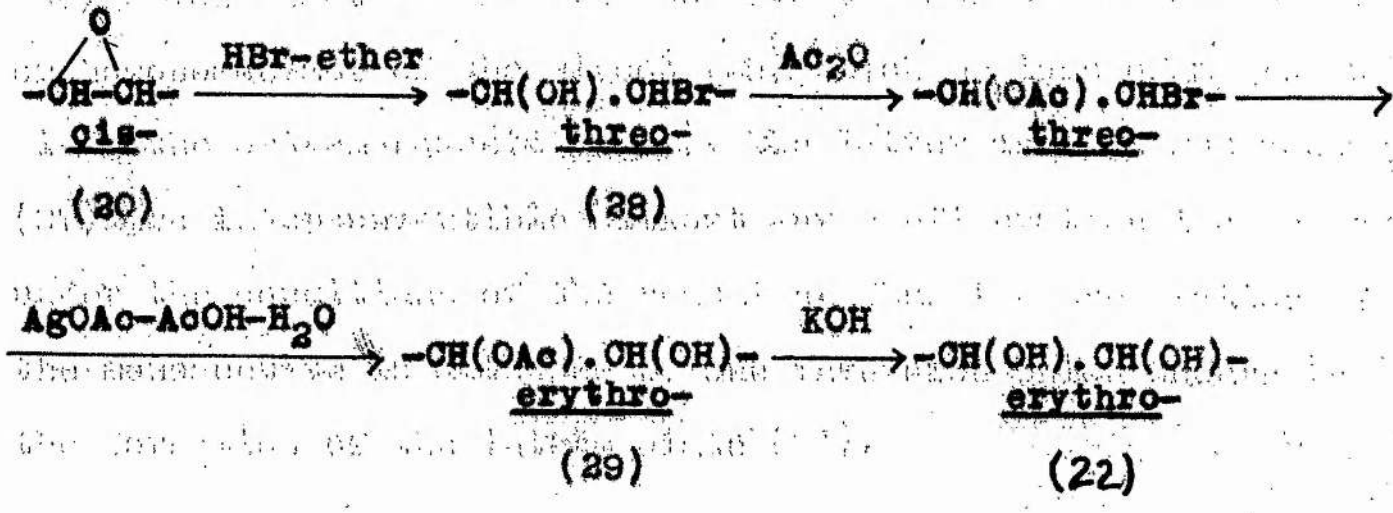
The reaction of these intermediates with a second inversion gives an apparent retention of configuration. Evidence for a similar chloronium intermediate is reported by Lucas and Gould

Presence of water in the solvent (acetic acid) during the reaction of silver acetate on acetoxybromobutanes gives a steri effect shifted towards inversion and the product is a monoacets With one equivalent of water complete inversion takes place and this has been explained by the formation of the intermediate (24) which reacts with water to give the conjugate acid of the orthomonoacetate of the glycol (25) which by loss of a proton gives the orthomonoacetate (26). The latter and the monoacetate (27) are interconvertible without any configurational changes under the conditions of the reaction. Thus the preparation of the monoacetate is attended by one inversion which occurs in the formation of the intermediate (24).



(24) (25) (26) (27)

cis- 12:13-Epoxyoleic acid (20) present in Vernonia anthelmintica[15] seed oil (also Cephalocroton cordofanus seed oil) was converted to threo- bromohydrin (28) by reaction with hydrogen bromide in ether, which after acetylation gave the erythro- monoacetate (29) by reaction with silver acetate in wet acetic acid and this on hydrolysis afforded the required erythro- 12:13-dihydroxyoleic acid (22).



The erythro- 12:13-dihydroxyoleic acid was a crystalline solid (m.p. 88°). The high overall yield of glycol resulting from this four-stage process (78% crude, 69% pure)- which also involves, in the final stage, removal of other acids originally present in the oil- and the ease with which the glycol is obtained pure are evidence of the high stereospecificity of these reactions. The procedure was later simplified by reducing the time of reaction from 8 to 2 hours, and also by effecting the reaction with silver nitrate in wet alcohol (cf. Bevan, Malkin and Smith[23]).

Hydrogenation of erythro- 12:13-dihydroxyoleic acid (m.p. 88°) gave erythro- 12:13-dihydroxystearic acid with a higher melting point (126°) than that previously recorded for the racemic- isomer (119-117°)[17]. This difference is due to the fact that our compound is an optically active isomer (discussed on page 140); the observed rotation is very small but larger for its unsaturated precursor.

The structures of threo- and erythro- dihydroxy acids were confirmed by oxidative studies now described.

threo-Dihydroxyoleic acid: This isomer has been proved by Gunstone[15a] to be 12:13-dihydroxyoctadec-9-enoic acid by oxidation with potassium permanganate and with potassium periodate.

erythro-Dihydroxyoleic acid: Oxidised with potassium permanganate in acetic acid at 45-50°, the erythro-dihydroxy-

unsaturated acid gave hexanoic and azelaic acids. When the saturated acid was similarly oxidised, hexanoic and dodecanedi acids were obtained. These results indicate that the acid is 12:13-dihydroxyoctadec-9-enoic acid.

Optical Activity:

It has already been mentioned that the two dihydroxyoleic acids (threo- and erythro-) are optically active. Since these have been prepared from epoxyoleic acid, the latter must be optically active and it has been shown that Cephalocroton codofanus seed oil exhibits small dextro rotation. (No value was measured for V. anthelmintica seed oil due to its dark colour.).

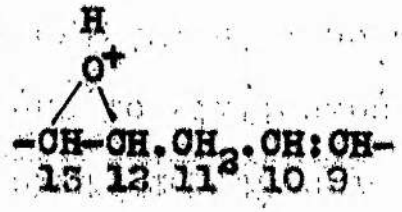
The optical rotation of the threo- and erythro-12:13-dihydroxyoleic acids is quite definite and easily measurable bu that of the saturated acids is very low, (see Table 2, p.147). This is not surprising as the optical activity of long-chain compounds is frequently so small that it cannot be measured and in the present work this difficulty was sometimes increased by the low solubility of the compounds. (Baer and Fischer[24] showed that certain triglycerides which should be optically active lack observable power but are optically active when one or more of the usual aliphatic acyl groups is replaced by an aromatic acyl group).

If the reaction mechanism involved in the preparation of erythro-12:13-dihydroxyoleic acid be represented as in Figure

(iv), it is seen that an enantiomorphic epoxide should give a active glycol.

On the other hand the active epoxide will give an active *threo*- glycol only if the conjugate acid (30) is attacked

(30)

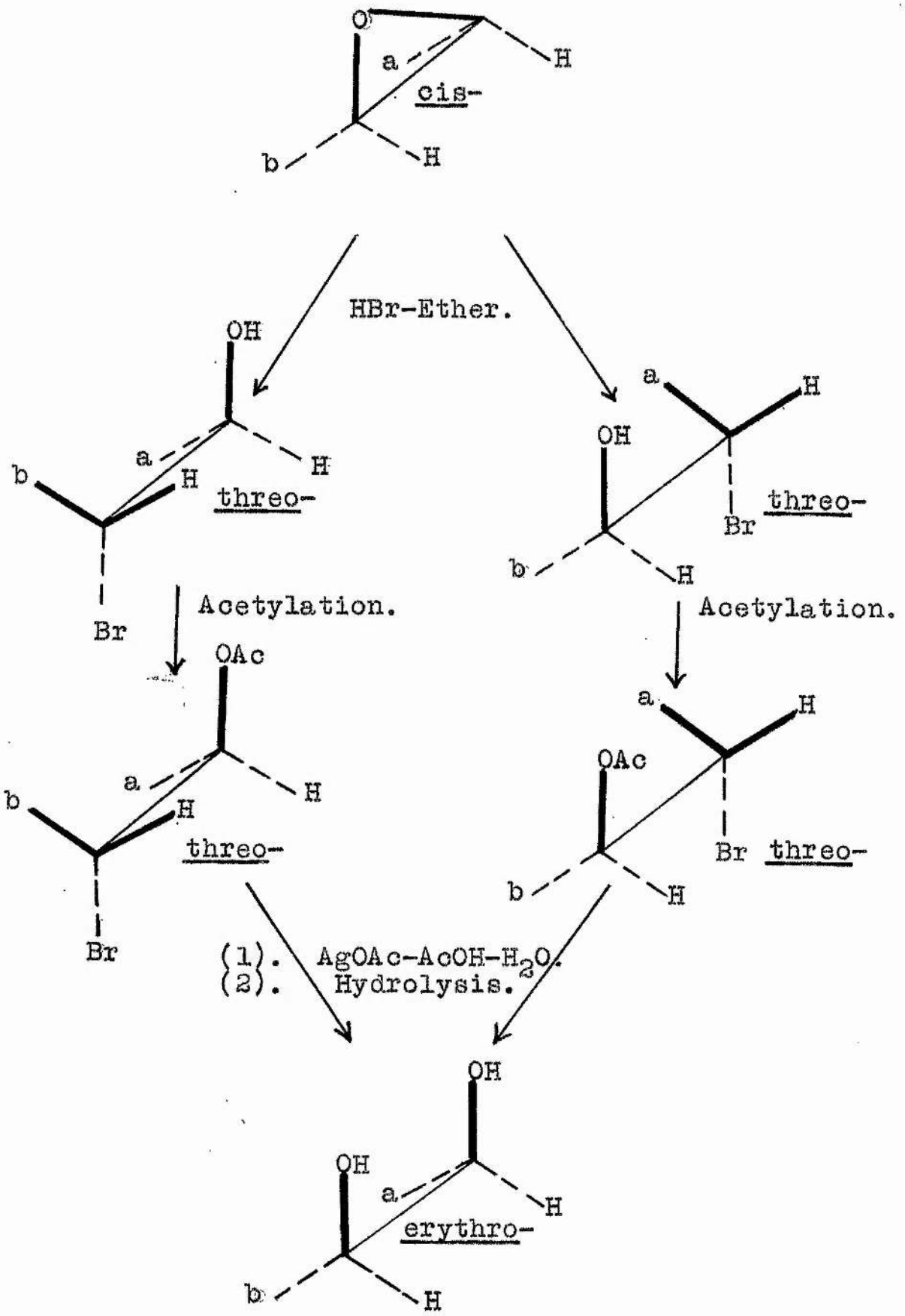


unequally at carbon atoms 12 and 13 by the acetate ion; an attack equally distributed between these two centres would give a *racemic*- glycol.

Although the optical rotation values for the two dihydro oleic acids (*threo*- and *erythro*-) are fairly small, the observed rotations are definite (see Table 3, page 147). The values for the corresponding saturated acids are less definit but as hydrogenation does not affect the active centres racemisation would not be expected to occur; further the melting point of the saturated *erythro*- acid differs appreci from that of the *racemic*- compound.

Oxidation of *threo*- and *erythro*- dihydroxyoleic acids with dilute alkaline permanganate or with performic acid will afford 9:10:12:13-tetrahydroxystearic acids and since the optically active centres (carbon atoms 12 & 13) will not be affected by these reagents (cf. ricinoleic acid[25]) the resulting tetrahydroxystearic acids should be optically activ

Figure (iv)


O
a
H
cis-
b
H
HBr-Ether.
OH
a
H
b
H
threo-
Br
a
H
OH
Br
threo-
b
H
Acetylation.
Acetylation.
OAc
a
H
b
H
threo-
Br
a
H
OAc
Br
threo-
b
H
(1). AgOAc-AcOH-H2O.
(2). Hydrolysis.
OH
OH
a
H
erythro-
b
H

Preparation of 9:10:12:13-Tetrahydroxystearic Acids.

The threo- and erythro- 12:13-dihydroxyoleic acids were oxidised with dilute alkaline permanganate by the method of Lapworth and Mottram[26] and with performic acid by Swern's procedure[27]. When the threo- dihydroxyoleic acid was oxidised with performic acid, the main product was a low-melting ether soluble compound which accompanied the desired tetrahydroxystearic acids. Two individual compounds (m.p. 77.5-78.0, 94-9 were isolated but not identified. Other investigators[8, 14] ha isolated similar compounds and furan compounds are reported[28] to be formed during the hydrolysis of 1:2:4:5-diepoxypentane. However, when the oxidation was repeated after acetylation of the hydroxyl groups, no low-melting products were formed, and hence performic acid oxidation was always carried out after acetylation of the starting material.

Each oxidation gave a mixture which was separated by an extensive series of crystallisations. The higher-melting isom being less soluble was generally more easily isolated whereas the lower-melting isomer was difficult to purify. Hence the homogenity of the latter is somewhat doubtful though in all cases the products were crystallised to constant melting poin McKay and his colleagues[14] report that the two tetrahydroxystearic acids are more easily separated as their methyl ester In some cases the results were checked by separation of both acids and their methyl esters with subsequent hydrolysis of t separated esters.

General Conclusions.

(1). The threo- and erythro- 13:13-dihydroxyoleic acids use for the preparation of tetrahydroxystearic acids are both crystalline solids easily purified by crystallisation and available in quantity. The use of these pure starting materia is an improvement over the work of other investigators who ha either used a concentrate of natural linoleic acid or α-linol acid obtained by debromination of tetrabromostearic acid. In the former case dihydroxystearic acid is formed from any olei acid initially present and this adds to the difficulty of isolating pure tetrahydroxystearic acids. In the latter case isomeric tetrahydroxystearic acids are formed as α-linoleic acid is known[13a] to be contaminated with small quantities of conjugated isomers and larger proportions of geometrical isomers. Thus the desired acids are contaminated with closely related compounds thereby increasing the difficulty of isolating pure substances.

The use of pure starting materials has resulted in high yields of separated tetrahydroxystearic acids as is apparent from Table 3 (page 159). The yields are higher than those reported by McKay and Bader[13] who have isolated threo- and erythro-9:10-dihydroxyoctadec-12-enoic acids as intermediates These are isomeric with the threo- and erythro-12:13-dihydrox octadec-9-enoic acids used in the present work and it is reasonable to assume that they should be solid. However,

McKay and Bader[13] obtained only the erythro- acid as solid, the other being a liquid and it is therefore considered unlikely that this liquid was pure.

(2)*. It has already been mentioned that the tetrahydroxystearic acids prepared during the present investigation are optically active. These are considered to be so for three reasons. The starting materials are active and it is believed that oxidation will not affect the asymmetric centres present as oxidation of ricinoleic acid gives optically active 9:10:13-trihydroxystearic acids[25]. Several of our tetrahydroxystearic acids show significant optical rotation (this refers to the observed rotation and not the calculated specific rotation). Those which do not could still be active as the optical activity of long-chain compounds is frequently so small that it cannot be measured. In some cases there is a marked difference in melting point between the active and inactive forms as is apparent from methyl erythro-9:10-erythro-12:13-tetrahydroxystearate. The active form melts at 171° whereas the racemate melts at 157°; the latter figue of McKay[14] was confirmed.

(3). The melting points of active and inactive isomers of long-chain compounds are frequently very similar and it is hence interesting to relate the present results with those of Kass and Burr[10], which have been questioned by McKay and Bader. Kass and Burr prepared two tetrahydroxystearic acids (m.p. 156-158° and 136-127°) by oxidation (with dilute alkaline permangana

* See Table 2, page 147 for Specific Rotations.

of a liquid isomer obtained during elaidinisation of linoleic acid. This isomer was considered to be the trans-9-cis-12-acid and the tetrahydroxystearic acids would hence be threo-9:10-erythro-12:13- acids. McKay and Bader, however, report 164° and 142° for these acids and suggest that Kass and Burr's products are the erythro-9:10-threo-12:13- acids by similarlity with the melting point of their acids (164°, 126°), which would be derived from the cis-9-trans-12-octadecadienoic acid. On the other hand our results indicate that Kass and Burr's original suggestion may be correct, but since our products are optically active and theirs racemic, this is not certain.

(4). Raman[15c] has confirmed the occurrence of cis-12:13-epoxy oleic acid in Vernonia anthelmintica seed oil and has prepared three tetrahydroxystearic acids therefrom. By performic acid oxidation of threo-12:13-diacetoxyoctadec-cis-9-enoic acid, two isomers, m.p. 146-148° and 123-125° were obtained. These must obviously be the threo-9:10-threo-12:13- acids and the melting points compare favourably with our values (148° and 122°). From alkaline potassium permanganate oxidation of the threo-12:13-dihydroxyoleic acid, a tetrahydroxystearic acid, m.p. 162-164° was obtained. This must be erythro-9:10-threo-12:13-acid by analogy with present work (cf. our value 164-165°). The latter acid (m.p. 164°) has also been obtained by Hopkins[16]. The lower melting erythro-9:10-threo-12:13- acid was not isolated by these workers[15c, 16]. We obtained this acid (111-113°) only after considerable difficulty and in poor yield.

TABLE 2. *Specific rotation.*

Acid	M. p.	Solvent: AcOH	Solvent: EtOH	M. p. of Me ester	Solvent: AcOH	Solvent: EtOH
threo-12 : 13-Dihydroxyoleic		**−6·8°** (19°, −0·69°)	**−5·7°** (19°, −0·51°)		—	—
erythro- ,, ...		**+1·2** (20, +0·12)	**−3·6** (20, −0·29)		—	—
threo-12 : 13-Dihydroxystearic.....		+1 (15·5, +0·01)	**−1·3** (20, −0·05)		—	—
erythro- ,, ...		+3 (16·5, +0·02)	−1·7 (20·5, −0·03)		—	—
Tetrahydroxystearic acids :						
threo-9 : 10-*threo*-12 : 13-	148°	—	—		—	—
,, ,, ...	122	−9 (17, −0·04)	0		0°	0°
erythro-9 : 10-*threo*-12 : 13-	165	−6 (20, −0·01)	—	136°	0	−1 (19, −0·01°)
,, ,, ...	112	—	—	—	—	—
threo-9 : 10-*erythro*-12 : 13-	156	**−44** (18·5, −0·35)	−7 (20, −0·03)	145	**−46** (18·5°, −0·09°)	—
,, ,, ...	130	**+14** (17, +0·10)	**+5** (20, +0·03)	114	**+17** (20, +0·12)	**+22** (20, +0·08)
erythro-9 : 10-*erythro*-12 : 13-	177	**−10** (18·5, −0·04)	+6 (20, +0·01)	171	**−16** (18·5, −0·08)	**+22** (20, +0·05)
,, ,, ...	156	+14 (17, +0·02)	0	145	0	−2 (19, −0·01)
				Cephalocroton cordofanus seed oil	**+3·4** (16·5, +0·08)	—

Measurements were made with a 2 dm. tube and sodium light.

A dash (—) indicates that no measurement was made, 0 indicates a zero value.

Values in heavy type are calculated from observed rotations of 0·03° or greater (some inactive dihydroxy- and tetrahydroxy-stearic acids gave observed rotations of only 0·00° or 0·01°); figures in parentheses are temperature and the observed rotation.

Experimental

15a

Preparation of threo-12:13-Dihydroxyoctadec-9-enoic Acid.

Vernonia anthelmintica seed oil or Cephalocroton cordofanus seed oil was boiled with glacial acetic acid (5-7 ml./g.) for 5 hours. About 2/3 of the acetic acid was removed distillation, the residue diluted with water and extracted with ether. The product was saponified with alcoholic potassiu hydroxide (1 N) and the mixed acids obtained by acidification and extraction with ether. 12:13-Dihydroxyoleic acid (m.p. 52-55°) was isolated by crystallisation from ether-petroleum ether (b.p. 40-60°, 1:1) and then from ethyl acetate. The overall yield was 57 % based on the epoxyoleic acid content of the oil

Partial Synthesis of Octadec-trans-12-enoic Acid.

(a). Hydrogenation of threo-12:13-Dihydroxyoleic Acid.

6.5 G. of dihydroxyoleic acid (m.p.52-55°) was dissolved in 60 ml. of ethanol and hydrogenated over 5% palladium-charcoal catalyst (0.3 g.). The catalyst was filtered off and the alcohol removed. The crude product after two crystallisati from chloroform (5ml./g.) gave 5.7 g. of threo-12:13-dihydroxy stearic acid, m.p. 95-96°.

(b). Preparation of erythro-12:13-Dibromostearic Acid.

5 G. of threo-dihydroxystearic acid (from a) dissolved in 50 ml. of hydrogen bromide in acetic acid (d = 1.25) and 5 ml. of concentrated sulphuric acid was left overnight at room

temperature. The solution was then heated to 100°C. for 8 hrs. further hydrogen bromide reagent (5 ml.) being added after 4 hours. The solution was cooled, diluted with water and extracted with petroleum ether (b.p. 60-80°). 6.73 G. of crude dibromostearic acid was obtained which on crystallisation from petroleum ether (b.p. 40-60°) gave 5.13 g. (74% yield) of pure acid melting at 47.5-48.5°. (Found: C, 49.0; H, 7.4; Br, 36.4 . $C_{18}H_{34}O_2Br_2$ requires C, 48.9; H, 7.75; Br, 36.1%).

(c). Debromination.

Zinc dust (8.7 g.), methanol (45 ml.) and aqueous hydrogen bromide (50%, 0.8 ml.) were refluxed for 5 minutes and then 4 g. of the dibromostearic acid was added and the mixture boiled for 1 hour, in a nitrogen atmosphere. The mixture was filtered, the solution diluted with water and extracted with ether (2 x 75 ml.). The ether extract after washing with 2 N aqueous potassium hydroxide solution (3 x 20 ml.) contained only 0.07 g. of material showing that practically no esterification had occured. The alkaline solution was acidified and extracted with ether and gave 1.66 (65 % yield) of crude octadec-trans-12-enoic acid. Crystallisations from methanol and from acetone gave a pure sample, m.p. 52-53° (lit. 52-53°)[17].

(Found: C, 76.6; H, 12.1. Calculated for $C_{18}H_{34}O_2$: C, 76.5; H, 12.1%).

Preparation of racemic-threo-12:13-Dihydroxystearic Acid.

0.44 G. of octadec-*trans*-12-enoic acid dissolved in water (44ml.) containing sodium hydroxide (0.44 g.) was diluted with water (350 ml.) and then oxidised at 10°C. with potassium permanganate solution (1%, 35 ml.). After 5 minutes the soluti was decolorised with sulphur dioxide, acidified with conc. hydrochloric acid (13 ml.) and left overnoght. 0.32 G. of crud dihydroxystearic acid was obtained which was purified by a single crystallisation from chloroform giving 0.16 g. of *racemic-threo*-12:13-dihydroxystearic acid, m.p. 96-97°, (lit.[17] 96.5-97.0°). [Mixed m.p. with *active-threo*-12:13-dihydroxystearic acid (m.p. 96-97°) was 95.5-96.0°].

Preparation of racemic-erythro-12:13-Dihydroxystearic Acid.

0.44 G. of octadec-*trans*-12-enoic acid was dissolved in 1.3 ml. of formic acid (98%) and 0.22 ml. of hydrogen peroxide (100 vol.) was heated for 2 hours at 40°C. The solution was then boiled for 1 hour with 25 ml. of aqueous 5 N sodium hydroxide solution, acidified with 5 N hydrochloric acid and the solids filtered, washed and dried. 0.45 G. of crude acid so obtained after crystallisation from ethyl acetate gave 0.37 g. of pure acid, m.p. 117-118°, (lit.[17] 119-120°). [Found: C, 68.1; H, 11.5 . Calculated for $C_{18}H_{36}O_4$: C, 68.3; H, 11.5%].

Preparation of erythro-12:13-Dihydroxyoctadec-9-enoic Acid.

Experimental details for unsuccessful attempts (A - C, pages 134-136) are not given and full details for the successful experiment D (page 136) are as follows:

53.G. of Cephalocroton cordofanus seed oil dissolved in 1500 ml. of ether saturated with hydrogen bromide was left overnight at room temperature and the solution was then washe with water, dried,and the solvent removed. 62 G. of bromohydri so obtained was acetylated by boiling with 300 ml. of acetic anhydride for 3 hours followed by 200 ml. of water for 30 minutes. The solution was cooled, diluted with water and extracted with ether giving 84 g. of acetoxy bromide. A solution of 20.8 g. of potassium acetate in 75 ml. of water was added to one of 32.8 g. of silver nitrate in 75 ml. of water, the precipitated silver acetate filtered on a Buchner funnel, washed with cold water and then with acetic acid. The silver acetate was added to 59 g. of acetoxy bromide dissolve in 325 ml. of glacial acetic acid containing 3.3 ml. of water and the mixture was refluxed for 8 hours. The cooled filtered solution was diluted with water and then extracted with ether. 54 G. of the product was obtained from the dried ethereal extract which was saponified with 1500 ml. of alcoholic potassium hydroxide solution (1 N) and the mixed acids recovered after acidification by extraction with ether. 45 G. of mixed acids so obtained on crystallisation from ether gave

24.1 g. (78% yield) of crude erythro- acid which after further crystallisation from ether gave 21.3 g. (69% yield) of pure erythro-12:13-dihydroxyoleic acid, m.p. 87-88. (Found: C, 68. H, 10.8 . $C_{18}H_{34}O_4$ requires C, 68.75; H, 10.9%).

The reaction proceeded equally smoothly when Vernonia anthelmintica seed oil was used. In another experiment when th time of reaction was reduced from 8 to 2 hours, the yield of the erythro-acid increased (82%).

Preparation of erythro-12:13-Dihydroxyoleic Acid Using Silver Nitrate in Wet Alcohol.

2.34 G. of acetoxy bromide (V. anthelmintica seed oil) wa prepared as already described and dissolved in 32.4 ml. of ethanol containing 3.4 ml. of water and 2.34 g. of finely powdered silver nitrate, and the mixture was refluxed for 1 hour. The insoluble salts were filtered off, the solution diluted with water and extracted with ether. The ether extract was washed with distilled water till free of inorganic salts, dried and evaporated. The residue was saponified with 50 ml. of alcoholic potassium hydroxide solution (1 N) for 1 hour and the mixed acids prepared. One crystallisation of the mixed acids from ether gave 0.66 g. of pure erythro-12:13-dihydroxy-oleic acid, (45% yield).

Derivatives of erythro-**12:13-Dihydroxyoctadec-9-enoic Acid.**

The following derivatives were prepared by standard procedures.

Methyl ester, m.p. 56-57.5°. (Found: C, 69.2; H, 11.2. $C_{19}H_{36}O_4$ requires C, 69.5; H, 11.1%).

Ethyl ester, m.p. 52-53°. (Found: C, 70.1; H, 11.3. $C_{20}H_{38}O_4$ requires C, 70.1; H, 11.2%).

p-Bromophenacyl ester, m.p. 116-117°. (Found: C, 61.1; H, 7.6; Br, 15.8 . $C_{26}H_{39}O_5Br$ requires C, 61.1; H, 7.7; Br, 15.6%).

Derivatives of erythro-**12:13-Dihydroxystearic Acid.**

When hydrogenated over 5% palladium-charcoal catalyst in ethanol, the erythro-12:13-dihydroxyoleic acid (100 mg.) gave erythro-12:13-dihydroxystearic acid (90 mg.), m.p. 125-126°, raised only to 125.5-126.5° after three crystallisations from ethanol and from ethyl acetate. (Found: C, 68.3; H, 11.3 . $C_{18}H_{36}O_4$ requires C, 68.3; H, 11.5%).

The following esters were prepared.

Methyl ester, m.p. 102-103°. (Found: C, 69.2; H, 11.6 . $C_{19}H_{38}O_4$ requires C, 69.1; H, 11.6%).

Ethyl ester, m.p. 97-98°. (Found: C, 69.7; H, 11.5 . $C_{20}H_{40}O_4$ requires C, 69.7; H, 11.7%).

p-Bromophenacyl ester, m.p. 136.5-137.5°. (Found: C, 60.6; H, 8.1; Br, 15.5 . $C_{26}H_{41}O_5Br$ requires C, 60.8; H, 8.1; Br, 15.6%).

Proof of Structure of erythro-13:13-Dihydroxyoctadec-9-enoic Acid.

(a). 1.8 G. of erythro-dihydroxyoleic acid was dissolved in 30 ml. of purified acetic acid[39] and 5.5 g. of finely powdered potassium permanganate was added portionwise so that the temperature did not exceed 50°. The mixture was maintained for 3 hours at 45-50°, a part of the acetic acid was then removed under reduced pressure, the product was suspended in 300 ml. of water containing dilute sulphuric acid and the mixture was decolourised with sulphur dioxide. The resulting mono- and di-basic acids were separated by steam distillation and extracted with ether from the volatile and non-volatile portions respectively.

The steam distillate (500 ml.) was extracted with ether (5 x 100 ml.) giving 0.59 g. of a monobasic acid after purification by distillation. This was identified as hexanoic acid, (p-bromophenacyl ester, m.p. and mixed melting point 70.5-71.5°, 63-64° when mixed with the ester of heptanoic acid).

The steam distillation residue (300 ml.) when extracted with ether (5 x 100 ml.) gave 1.16 g. of an acid. This was extracted with boiling water (60 ml.) and filtered from the insoluble oil. From the filtrate 0.59 g. of crude azelaic acid was obtained and purified by one crystallisation to an acid, melting point and mixed melting point 105-106°.

(b). 1.01 G. of erythro-dihydroxystearic acid was oxidised

with 3 g. of potassium permanganate in 20 ml. of acetic acid, and the products of oxidation were separated into two portions by steam distillation as in (a). From the steam volatile porti 0.16 g. of hexanoic acid was isolated and identified , (p-brom phenacyl ester, m.p. and mixed m.p. 71.5-72.0°; 62-63° when mixed with the ester of heptanoic acid). The non-volatile material gave 0.23 g. of a dibasic acid identified as dodecanedioic acid, m.p. and mixed m.p. 129-131°; 112-114° when mixed with sebacic acid.

The isolation and identification of hexanoic, azelaic, an dodecanedioic acids indicate that the acid is 12:13-dihydroxy octadec-9-enoic acid.

[The structure of the threo-dihydroxyoleic acid has been proved by Gunstone [15a].

Preparation of 9:10:12:13-Tetrahydroxystearic Acids.*

erythro-9:10-threo-12:13-Tetrahydroxystearic Acids (14 & 15).

5 G. of threo-12:13-dihydroxyoleic acid was dissolved in 500 ml of water containing 5 g. of sodium hydroxide and diluted with 4 litres of water. The solution was cooled to 10°C and oxidised by the slow addition of 550 ml. of 1 % potassium permanganate solution. After 5 minutes the solution was decolourised with sulphur dioxide, acidified with 150 ml. of concentrated hydrochloric acid and then left overnight at room temperature. The mixture was filtered and the solids washed with a little water, dried and extracted with petroleum ether (b.p. 40-60°). 3.36 G. of crude tetrahydroxystearic acids were thus obtained.

1.6 G. of the crude material was separated into the two isomers by utilising the lower solubilty of the higher melting acid in ethyl acetate. This after crystallisation from ethanol gave 0.44 g. of pure acid, m.p. 164-165°. Its methyl ester, m.p 135.5-136.0° was prepared by methylation with methanolic hydrogen chloride. From the ethyl acetate soluble portion the lower melting isomer was isolated which after repeated crystallisation from aqueous alcohol, ethyl acetate and finally from acetone gave 0.16 g. of an acid, m.p. 111-113°. The general scheme of separation is shown in Figure (v).

* [See Table 2 for Specific rotation of these acids (page 147) and Table 3, (page 159) for analyses and yields of the tetrahydroxystearic acids].

TABLE 3. 9 : 10 : 12 : 13-*Tetrahydroxystearic acids.*

	threo-9 : 10-*threo*-12 : 13- **(18,19)**		*erythro*-9 : 10-*threo*-12 : 13- **(14,15)**		*threo*-9 : 10-*erythro*-12 : 13- **(16,17)**		*erythro*-9 : 10-*erythro*-12 : 13- **(12,13)**	
Yield of mixed tetrahydroxystearic acids (%)	82		61		92		59	
Yield of separated acids :								
(i) separated as acids (%) *	8	34	17	6	21	41	24	13
(ii) separated as esters (%) *	—	—	20	1·1	25	25	26	17
Acid, m. p.	147·5—148·5°	121—122°	164—165°	**112—113°**	**156·5—157°**	**129·5—131°**	176·5—177°	**155·5—156·5°**
C (%) †	62·1	62·0	62·0	62·2	62·1	61·9	62·2	62·1
H (%) †	10·5	10·3	10·6	10·1	10·6	10·6	10·4	10·5
Methyl ester, m. p.	—	—	135·5—136°	—	144·5—145·2°	113·5—115°	**170·5—171°**	143—146°
C (%) ‡	—	—	62·8	—	62·8	63·0	62·8	63·1
H (%) ‡	—	—	10·3	—	10·6	10·8	10·7	10·3
Racemic compound : [13, 14]								
acid, m. p.	148°	126°	164°	126°	164°	142°	174°	164°
ester, m. p.	118°	95°	—	—	—	—	157°	145·5°

* Based on the total theoretical yield.

† $C_{18}H_{36}O_6$ requires C, 62·0, H, 10·4%.

‡ $C_{19}H_{38}O_6$ requires C, 63·0; H, 10·6%.

M. p.s in heavy type differ appreciably from those quoted for the racemic compound.

The remainder of the mixed acids (1.7 g.) was methylated with methanolic hydrogen chloride and the methanol solution c progressive concentration gave several fractions of the highe melting isomer (0.58 g.), which on crystallisation from methanol melted at 135-135.5°. From the mother liquors a smal amount of a sticky solid was obtained which on hydrolysis gav the acid melting at 111-113°. [see Figure (vi)].

Figure (v).

Separation of mixed erythro-9:10-threo-12:13-Acids.

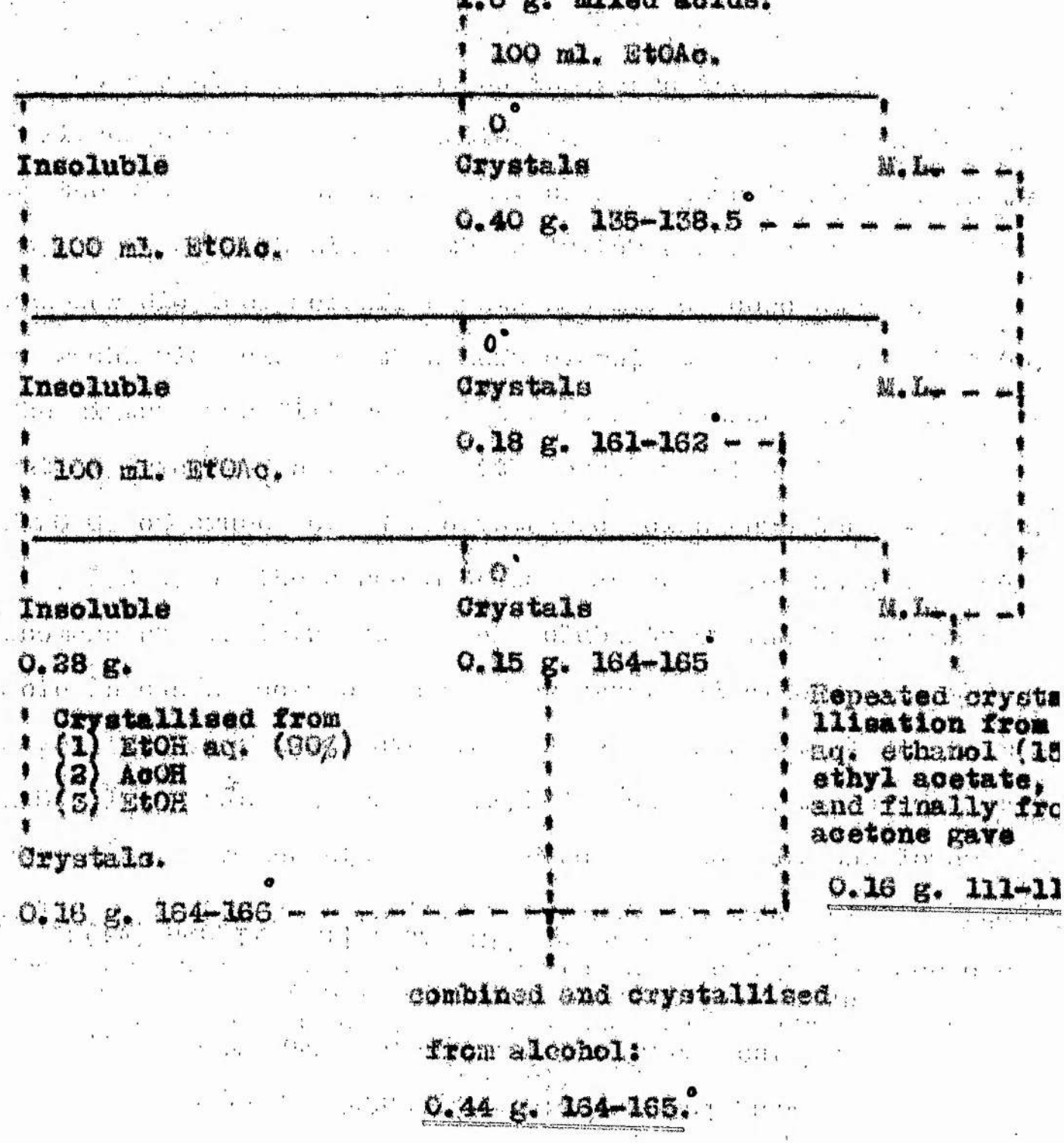
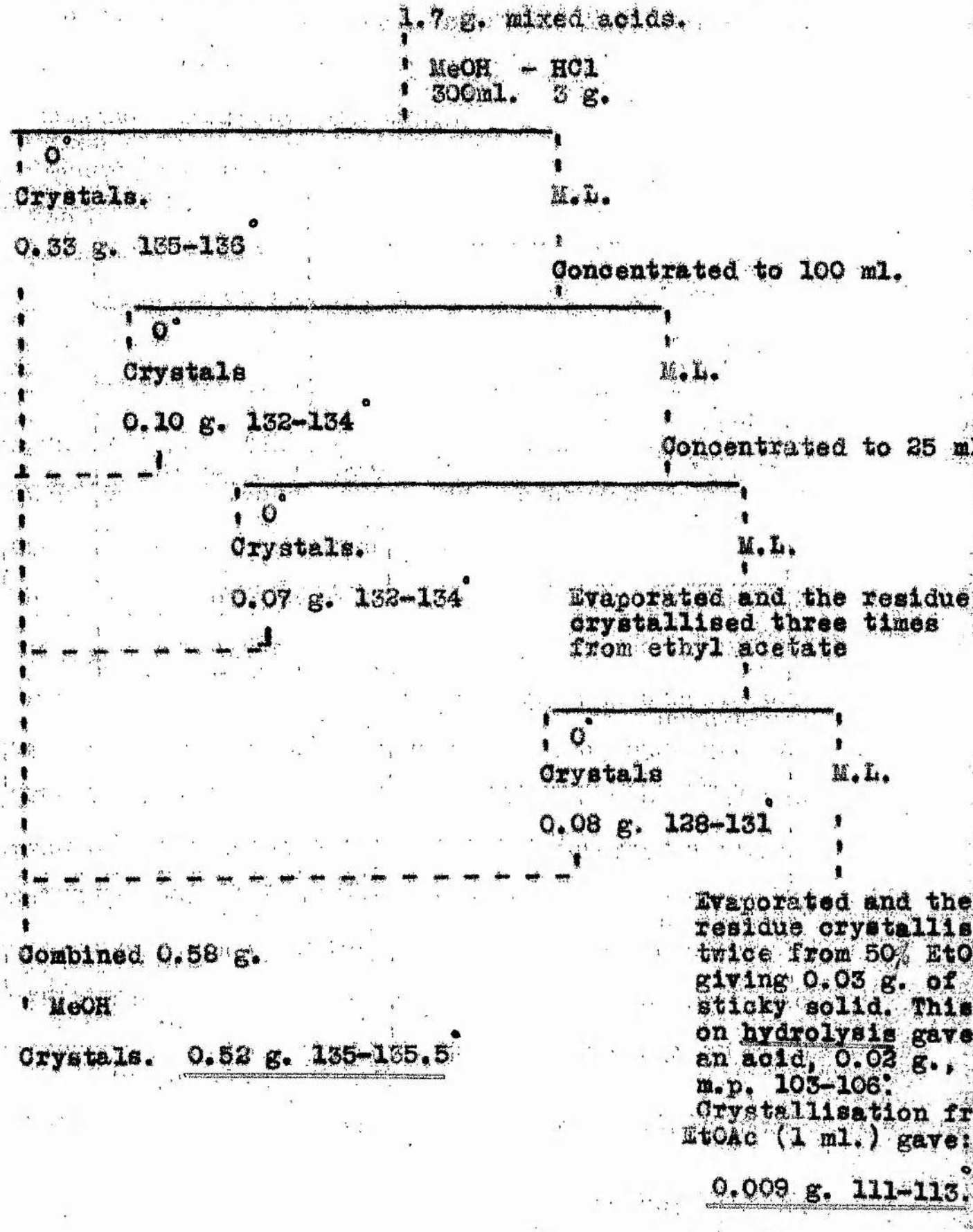


Figure (vi).

Separation of mixed erythro-9:10-threo-12:13- esters.



threo-9:10-threo-12:13-Tetrahydroxystearic Acids (18 & 19).

6 G . of threo-12:13-dihydroxyoleic acid was acetylated by boiling with 50 ml. of acetic anhydride for 4 hours and then for a further 2 hours after addition of water. Extraction with ether gave 7.23 g. of the diacetoxyoleic acid . (The original dihydroxyoleic acid could be recovered in good yield by alkaline hydrolysis - 0.46 g. from 0.54 g. of the acetylate acid.) 5.4 G. of the diacetoxyoleic acid was dissolved in 13 m of formic acid (98 %) and 2 ml. of hydrogen peroxide (100 vols The solution was kept for 2 hours at 40° and a part of the formic acid was removed under reduced pressure. The residue wa hydrolysed by boiling with 90 ml. of aquwous sodium hydroxide solution (3N) for 1 hour and the tetrahydroxystearic acids liberated by acidification with hydrochloric acid (3N). 4.54 g of crude material was obtained after washing and drying. Two isomers, 0.40 g., m.p. 144-146° and 1.59g., m.p. 121-122° were obtained by fractional crystallisation as shown in Figure (vi

When the dihydroxyoleic acid (5 g.) was similarly oxidise without initial acetylation, the crude product (4.6 g.) gave a large ether-soluble fraction (2.53 g., m.p. 63-68°) from which two products were isolated by fractional crystallisation but not identified: (a) m.p. 77.-78.5° (Found: C, 65.2; H, 10.1 Calculated for $C_{18}H_{34}O_5$ C, 65.4; H, 10.4%) and (b) m.p. 94-9 (Found: C, 65.1; H, 10.0%). The compound (b) depressed the m. of Compound A^{14} (m.p. 95.5-96.0°) to 82-84° and of compound B^{14} (m.p. 90-91°) to 81-83°.

Figure (vii)

Separation of Mixed threo-9:10-threo-12:13-**Acids.**

4.54 g. of mixed acids.

2 x 50 ml. ether

Insoluble

3.87 g.

2 x 50 ml. acetone

Solution

Insoluble

2 x 50 ml. acetone

R.T. Crystals

2.12 g. 119-123°

M.L.

Several crystallisat from aceton and from eth acetate gave

1.59 g. 121-

Insoluble

2 x 50 ml. acetone

R.T. Crystals

0.64 g. 119-132°

M.L.

Insoluble

Trace.

R.T. Crystals

0.40 g. 144-146°

M.L.

Several crystallisations from acetone and from ethyl acetate raised the melting point to

147.5-148.5°

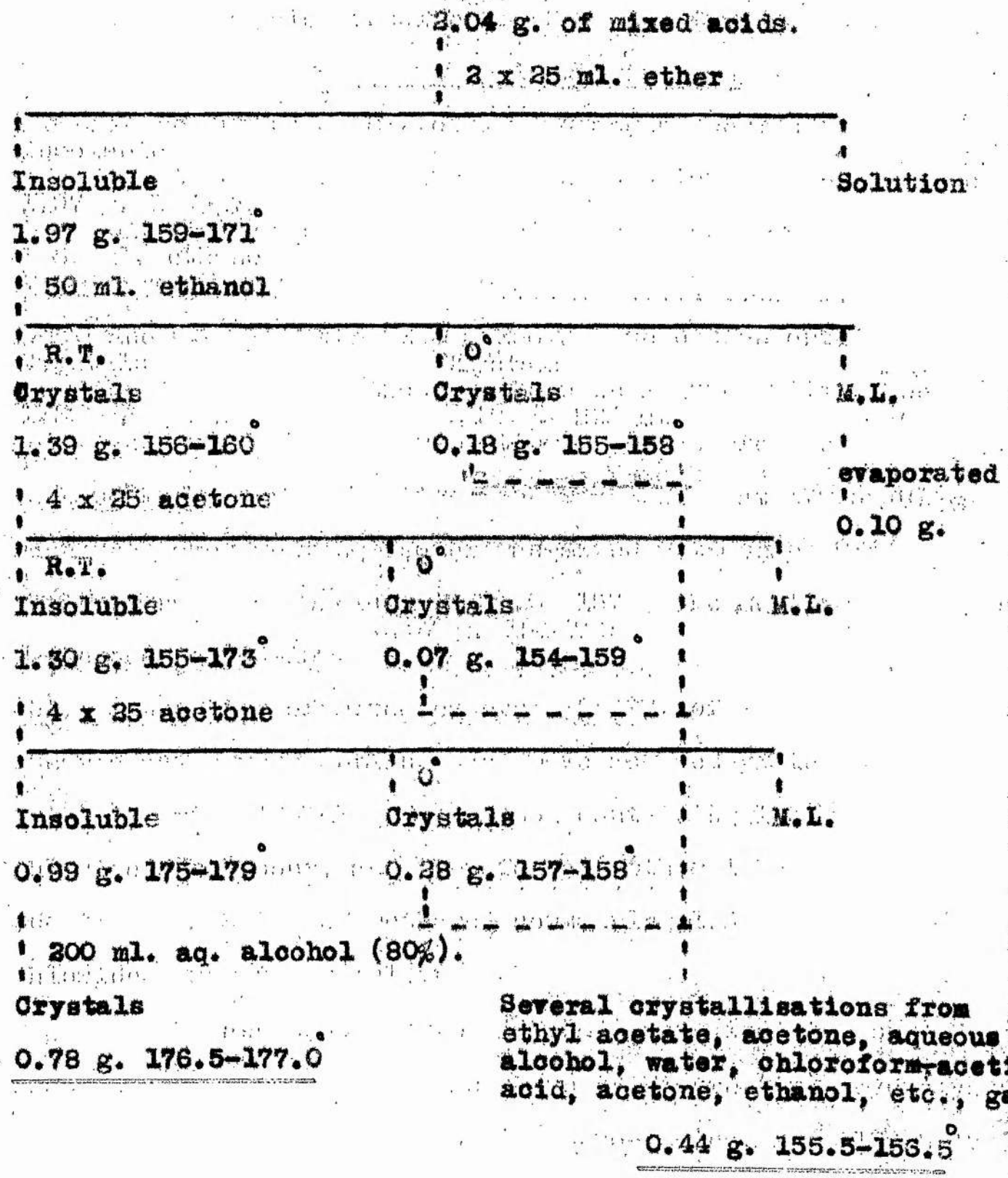
erythro-9:10-erythro-12:13-Tetrahydroxystearic Acids (12 & 13)

(a). Separation of mixed acids: 3 G. of erythro-12:13-dihydroxyoleic acid dissolved in 300 ml. of water containing 3 g. of sodium hydroxide was diluted with 2.4 litres of water and oxidised with 290 ml. of 1 % potassium permanganate solution at 10°. After 5 minutes the solution was decolourised with sulphur dioxide and when acidified gave 2.04 g. of crude tetrahydroxystearic acids. Two isomers, 0.78 g., m.p. 176.5-177.0° and 0.44 g., m.p. 155.5-156.5°, were then obtained by fractional crystallisation as shown in Figure (viii). The methyl ester of the higher melting acid was prepared by methanolic hydrogen chloride and this melted at 170.5-171.0°. When the lower melting isomer was mixed with threo-9:10-erythro-12:13-tetrahydroxyoleic acid, m.p. 157°, the melting point was depressed to 145-150°.

(b). Separation of mixed esters: 3.6 G. of mixed tetrahydroxystearic acids were obtained as above and methylated by refluxing with 135 ml. of methanol containing 1.3 g. of hydrogen chloride for 1 hour, and then for a further 1 hour after addition of 25 ml. of methanol containing 1.5 g. of hydrogen chloride. From the resulting solution two pure esters, 1.50 g. m.p. 169-171° and 0.96 g. m.p. 143-144°, were isolated by fractional crystallisation as shown in Figure (ix). Hydrolysis of these esters gave the two dierythro- acids, m.p. 176.5-177° and 155°, respectively.

Figure (viii)

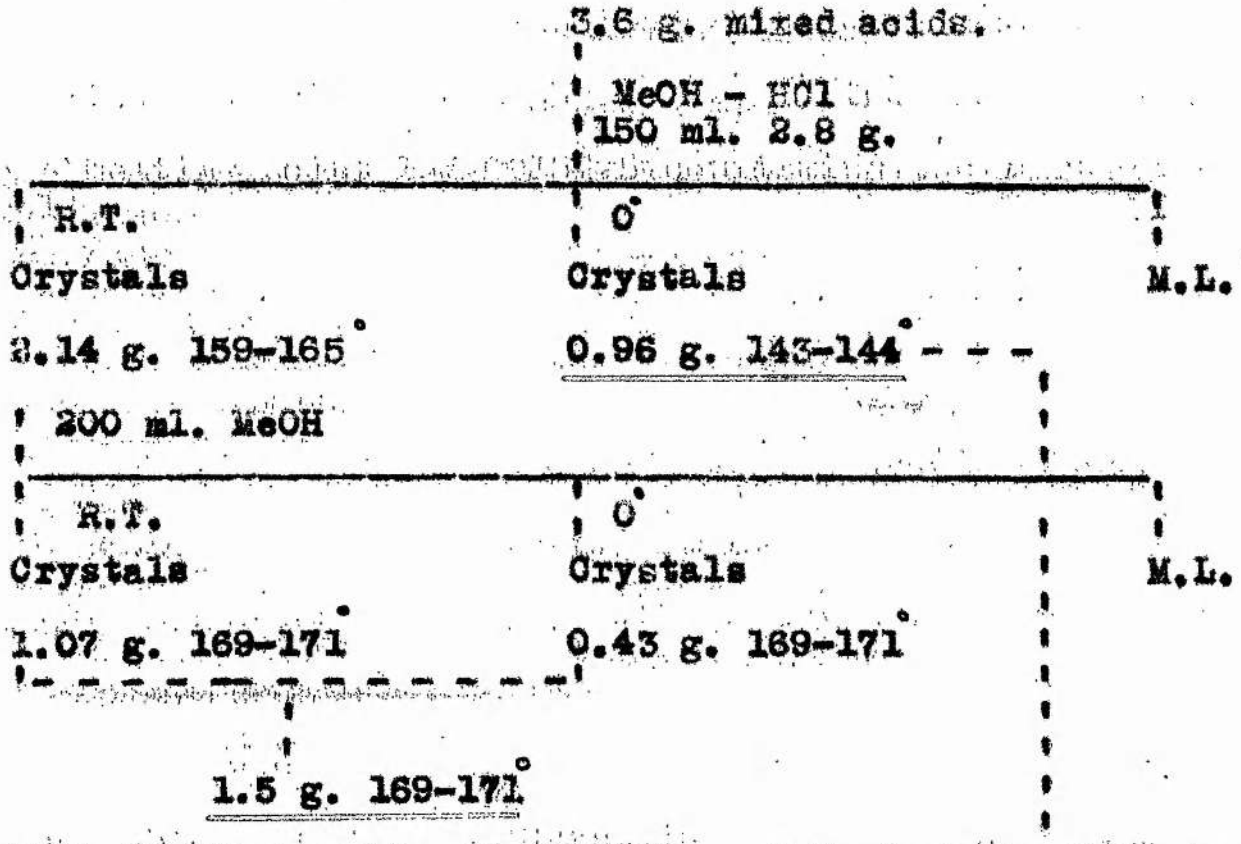
Separation of Mixed erythro-9:10-erythro-12:13- Acids.



(c). racemic-dierythro-Tetrahydroxystearic acids: The two racemic- dierythro- acids (m.p. 171.5-172.5° and 154-156°) were prepared by dilute alkaline potassium permanganate oxidation of linoleic acid. The methyl ester of the higher melting acid had melting point 157.0-157.5° confirming the value of McKay et al[14].

Figure (ix)

Separation of mixed erythro-9:10-erythro-13:13-Esters.



three-9:10-erythro-12:13-Tetrahydroxystearic Acids (16 & 17).

(a). Separation of mixed acids: 5 G. of erythro-12:13-dihydroxyoleic acid was acetylated and the product (6.23 g.) oxidised with performic acid (15 ml. of 98 % formic acid and 2.4 ml. of 100 vols. hydrogen peroxide) as already described. 5.13 G. of crude tetrahydroxystearic acids were obtained of which 5.03 g. were insoluble in ether. A portion of the acids (3.5 g.) was separated into pure isomers, m.p. 156.5-157.0° and 129-131° by fractional crystallisation as shown in Figure (x).

(b). Separation of mixed esters: The remainder of the mixed acids (2.52 g.) were esterified with cold methanolic hydrogen chloride. From the resulting solution, two esters, m.p. 144.5-145.5° and 113.5-115.0° were isolated as shown in Figure (xi). Hydrolysis gave the acids, m.p. 155.5-157.0° and 130-131°, respectively.

Figure (x)

Separation of Mixed threo-9:10-erythro-12:13-Acids.

8.5 g. of mixed acids.
50 ml. acetone

R.T.	
Insoluble	Crystals — M.L.
50 ml. acetone	0.42 g. 128-131°

R.T.	
Insoluble	Crystals — M.L.
50 ml. acetone	0.60 g. 125-131°

R.T.	
Insoluble	Crystals — M.L.
0.88 g. 148-152°	0.35 g. 129-131°
30 ml. ethyl acetate	

R.T.	
Insoluble	Crystals — M.L.
0.57 g. 155-158°	0.12 g. 129.0°

Repeated extraction with acetone and with ethyl acetate followed by crystallisations from ethanol gave an acid melting at

156.5-157.0°

1.27 g. 128-131°

After repeated crystallisation from ethanol and from ethyl acetate this acid melted at

129.5-131.0°

Figure (xi)

Separation of Mixed threo-9:10-erythro-13:13-Esters.

2.52 g. mixed acids.

MeOH - HCl
300 ml. 2.5 g.

Left at room temperature overnigh
nothing separated even when coole
to 0° for a few hours; hence the
solution was concentrated to 75 m

R.T.
Crystals
0.42 g. 143-146°

0°
Crystals
0.37 g. 142-145°

M.L.
concentrate
to 30 ml.

0.79 g. 142-145°

After crystallisation from acetone, from ethyl acetate and from methanol, the ester melted at

144.5-145.5°

Hydrolysis gave an acid

m.p. 155.5-157.0°

0°
Crystals
1.63 g. 90-116°

Crystallised from:
(1) acetone
(2) ethyl acetate
(3) methanol

Crystals
0.72 g. 113.5-115.0

Hydrolysis gave an acid

m.p. 130-131°

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VEGETABLE OILS. IV.*—A New Method of Determining the Component Acids of Oils containing Epoxy- and/or Hydroxy-Acids

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A new method of determining the component acids of oils containing epoxy- and/or hydroxy-acids is described and applied to the seed oils of *Vernonia anthelmintica* and *Strophanthus hispidus* containing 12 : 13-epoxyoctadec-9-enoic (74%) and 9-hydroxyoctadec-12-enoic acid (15%) respectively. Some new derivatives of this latter acid are reported and the periodate method of determining α-glycols is criticized.

Introduction

In previous publications[1, 2, 3] it has been shown that seed oils of the *Strophanthus* species differ from all other seed oils in that so far as is at present known they alone elaborate among their component acids 9-hydroxyoctadec-12-enoic acid isomeric with the more familiar ricinoleic acid (12-hydroxyoctadec-9-enoic acid). This discovery led to the re-examination of some other

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seed oils reported to contain hydroxy-acids and we have found[4] that the major component acid of *Vernonia anthelmintica* is not 11-hydroxyoctadec-9-enoic acid[5] but 12 : 13-epoxyoleic acid.

The usual method of component acid determination involves the separation of the mixed fatty acids by low-temperature crystallization into three fractions containing, respectively, mainly saturated, mono- and polyethenoid acids, followed after esterification by fractional distillation of each fraction. Determination of mean unsaturation, mean equivalent, and ultra-violet absorption after alkali-isomerization then suffices to characterize each of the relatively simple ester fractions. When this method was applied to various *Strophanthus* oils containing relatively small amounts (7–14%) of a monohydroxyoctadecenoic acid, certain modifications had to be made, mainly because this acid does not react quantitatively with Wijs reagent but gives values considerably higher than theoretical. This effect is reduced or eliminated by acetylation of the esters. In our previous analyses of *Strophanthus* oils, fractions containing the hydroxy-acid were methylated and acetylated before distillation and the proportion of methyl acetoxyoctadecenoate in the distilled ester fractions was computed solely from the saponification equivalent. This method which then appeared to be adequate might be less satisfactory in the presence of larger proportions of hydroxy-acid. In addition, the accuracy of the process is dependent on the thermal stability of the acetoxy-ester.

It is perhaps on account of this last factor that other methods of analysing castor oil (which contains high proportions of ricinoleic acid) have been described. In the most complete study of castor oil Gupta, Hilditch & Riley[6] did not separate the acids by low-temperature crystallization, as they found this process unsuitable for mixtures containing ricinoleic acid, nor were the esters distilled. The proportion of ricinoleic acid followed from the acetyl value determined by Riley's method[7] after making allowance for the dihydroxystearic acid weighed after crystallization ; ultra-violet absorption after alkali-isomerization gave the content of linoleic acid, whilst oleic acid was determined from the iodine value after allowance for the linoleic and ricinoleic acid ; the saturated acids were determined as a group by difference. This method of computing the content of oleic and of saturated acids would obviously be unsatisfactory in the presence of acids which react abnormally with Wijs reagent.

Another method of examining mixtures containing ricinoleic acid was described by Achaya & Saletore.[8] Saturated acids were first removed by lead salt separation and the remaining acids were then separated into two fractions by a technique in which oleic and a little linoleic acid formed a urea complex whilst ricinoleic and most of the linoleic acid did not. This last fraction was subsequently methylated, acetylated and distilled.

In our investigation of the seed oil of *V. anthelmintica*, the reactive nature of the epoxy-group greatly limited the processes available in any method of analysis ; in particular the acid could not be esterified by the usual methods as the epoxide ring reacts readily with methanolic hydrogen chloride or methanolic sulphuric acid.[4] This difficulty was avoided by converting the acid to the corresponding dihydroxy acid and this procedure has enabled us to devise a method of analysis suitable for oils containing monohydroxy-, dihydroxy- or epoxy-acids. In view of the difficulty of determining the iodine value of both 12 : 13-dihydroxyoleic (ref. 4, but see ref. 9) and 9-hydroxyoctadec-12-enoic acids,[1] separation of the hydroxy- and non-hydroxy-acids was tried using a method of distribution of the acids between two suitable immiscible solvents, as the two types of acids have different solubility characteristics. This technique has been successfully applied in the concentration of the hydroperoxide resulting from autoxidation experiments.[10, 11] This method of separation was found to be effective and a method based on it for the analysis of oils containing hydroxy- and/or epoxy-acids has been developed.

Experimental

The method of analysis

It will be convenient to describe the method in general terms before discussing its application in certain specific instances. This method was later modified slightly in the light of experience gained during its use.

The first step involves the preparation of mixed acids freed from unsaponifiable matter and

so treated, if epoxy-acids be present, that the latter are converted to dihydroxy acids. This is achieved by refluxing the oil with five volumes of acetic acid for 5–7 hours, after which the acetic acid is distilled off, the last traces under reduced pressure. The product (in which any epoxy-glyceride has been converted to monohydroxy-monacetoxy-glyceride) is hydrolysed with alcoholic potash and the unsaponifiable material removed in the usual way. The treatment with acetic acid is not required in the absence of epoxy-acids.

The hydroxy-acids are next separated from non-hydroxy-acids by partition between light petroleum (b.p. 40–60°) and methanol–water (4 : 1) which have previously been equilibrated by shaking together. Mixed acids containing a high percentage of hydroxy-acids are best dissolved in 80% methanol and extracted repeatedly with light petroleum, whilst acids containing smaller proportions of hydroxy-acid are dissolved in light petroleum and extracted with 80% methanol. The process is best illustrated by an actual example.

To 1 l. of 80% methanol in each of three separating funnels (1–3) was added about 50 g. of acids from *V. anthelmintica* seed oil ; 500 ml. of 80% methanol was placed in each of two further funnels (4–5). Light petroleum (250 ml.) was added to the first funnel and after equilibration passed to each of the other four funnels in turn. This was followed by other portions of light petroleum until little or no material was extracted from the methanol solutions ; acids remaining in the methanol solution were then recovered by distilling off most of the methanol, adding water, and extracting with ether. The distribution of the material was then as follows :

	Methanol solutions		Light-petroleum extracts				
No.	1 2 3	4 5	5	4	3	2	1
Wt., g.	113·8	4·3	1·1	1·8	3·6	9·9	21·3

These figures suggest that the separation of dihydroxy- from non-hydroxy-acids is readily effected ; later results indicate that the separation is fairly sharp.

The non-hydroxy-acids are then divided into two fractions by crystallization from methanol at — 20° (which is known to separate saturated from unsaturated acids[12]), or by the lead salt method. The original mixed acids are thus divided into three fractions in which saturated (A), unsaturated (B) and hydroxy-acids (C) are separately concentrated. It may be advantageous in some cases to effect the low-temperature crystallization before the light petroleum/methanol distribution.

Fractions A and B are further examined in the usual way, except that fraction B, which may contain small proportions of hydroxy-acids, is acetylated prior to distillation. When fraction C was methylated, acetylated and distilled there was evidence of slight decomposition of the diacetoxy-esters and accordingly we have analysed this fraction without distillation. From the saponification equivalent (determined in quadruplicate) of the methyl esters of this fraction before and after acetylation it is possible to determine the content of hydroxy-ester. These values are connected by the following expression :

$$\% \text{ Hydroxy ester} = \frac{100 \times M(\mathrm{B} - \mathrm{A})}{56{,}100n - \mathrm{B}(M' - M)}$$

(M and M' are the molecular weights of the hydroxy- and acetoxy-esters ; n is the number of hydroxyl groups present in the ester ; A and B are the observed saponification values before and after acetylation.)

This value was found to exceed 90%, leaving only a small proportion of unknown composition, and, in view of the difficulty of determining the iodine value of this fraction, we have assumed that the non-hydroxy-acids present have the same composition as the non-hydroxy-acids in fractions A and B together. Although this must introduce some element of doubt into the final results, we do not consider that the error will be very great. It seemed to us that the proportion of hydroxy-acid in fraction C (90–95%) should be higher, but we have shown that this value is not seriously low on account of incomplete acetylation (the value obtained after refluxing with acetic anhydride for 1 hour is hardly changed after 10 hours) and by the fact that pure dihydroxyoleic acid gives a value of 98·5% after refluxing for 2 hours (the recommended period).

At this point it is possible to calculate the composition of the mixed acids in terms of the

various non-hydroxy- and hydroxy-acids and it is then necessary for oils containing epoxy-acids to determine the proportion of epoxy-glyceride in the original oil by the method of King[13] using dioxan–hydrochloric acid reagent. This value expressed as a molecular percentage is compared with the amount of dihydroxy-acid similarly expressed. If the latter exceeds the former by more than the experimental error, then epoxy- and dihydroxy-acid must have been present originally. The method of calculation assumes the presence of only a single hydroxy- or dihydroxy-acid; experiments supporting this are described later for each oil.

This procedure has been successfully applied to *V. anthelmintica* seed oil containing over 70% of epoxyoleic acid, to *S. hispidus* seed oil which contains less than 20% of monohydroxyoctadecenoic acid, and to *Cephalocroton cordofanus* seed oil which has also been shown to contain epoxyoleic acid in high proportion.[14] We consider that the method may be usefully applied to oils containing epoxy-, monohydroxy- or dihydroxy-acids and, whilst there is a little more manipulation than in low-temperature crystallization, no special apparatus or materials are required.

Vernonia anthelmintica *seed oil*

The oil (Table I) was extracted from a further sample of the seeds previously used in the characterization of the epoxy-acid.[4] This was converted into mixed hydroxy-acids (Table I) by the methods already described and the mixed acids were distributed between light petroleum and aqueous methanol. The non-hydroxy acids were then crystallized from methanol at — 20°. At this point difficulty was encountered because of the unsaponifiable material present; this was therefore removed from fractions A and B. Fraction A was esterified with methanol and sulphuric acid, fractions B and C were esterified with methanolic hydrogen chloride and the whole of fraction B and a part of fraction C subsequently acetylated by boiling with acetic anhydride for 2 hours. Fractions A and B were then distilled and examined in the usual way, the small quantity of A esters being distilled through a small Widmer column. The saponification equivalent of fraction C esters was determined in quadruplicate before and after acetylation; unsaponifiable material in this fraction was measured quantitatively.[15]

The composition of fractions A and B was computed in the usual way; that of fraction C was calculated in terms of dihydroxyoleic acid, unsaponifiable material and non-hydroxy-acids (considered to have the same composition as in fractions A and B together). The results are summarized in Table I.

The absence of monohydroxy-acid was indicated by the following experiment. A sample of the fraction C acids was dissolved in 80% aqueous methanol and extracted with light petroleum in a continuous extractor for 16 hours. The extracted material (2·4 g., 5·8%) contained 56% of dihydroxy-acid as determined by the glycol value and a maximum of 49% as determined by the equivalent (single determination) of the ester before and after acetylation. The former value is not affected by the presence of mono-hydroxy-acids which would be concentrated in this extract if they were present.

The presence of all the acids listed in Table I has previously been confirmed[4] with the exception of arachidic and oleic acids; the oleic acid present in fractions A1 and A2 has now been identified by oxidation to 9 : 10-dihydroxystearic acid (m.p. and mixed m.p. 129–130°).

V. anthelmintica seed oil is of great interest in that it contains almost 90 mol.-% of epoxy-oleic and linoleic glycerides together. Dehydrated castor oil containing a high content of octadecadienoic glycerides has found extensive use as a drying oil; dehydration of epoxyoleic acid could lead to octadecatrienoic acid and dehydrated *V. anthelmintica* seed oil might have valuable drying properties. Experiments on this aspect of the matter are now in progress.

Strophanthus hispidus *seed oil*

Work on this oil was carried out for two purposes. Firstly to see how successfully the method developed for oils containing high proportions of dihydroxy-acids could be applied to oils containing low proportions of monohydroxy-acids and secondly to try to devise a quicker method of analysing *Strophanthus* oils.

The oil used was the same as that previously examined,[3] and the mixed acids after removal of unsaponifiable material (1·32%) were submitted to the following procedures.

Table I

V. anthelmintica *seed oil*

Characteristics	Oil	Mixed hydroxy-acids
Saponification equivalent	320·7	329·5
Iodine value (mean of two determinations)	101·7 ± 0·5	107·4 ± 1·5
Epoxyoleic glyceride (% wt.)	71·5	—
Absorption max. ($E^{1\%}_{1\,cm.}$) at 234 mμ after isomerization (180°/60 min.)	—	115·3*

* Measured on acids freed from unsaponifiable material

Separation of acids	%	Iodine value
Fraction A	3·8	24·4
Fraction B	13·5	149·0
Fraction C	75·8	88·9
Unsaponifiable material from A and B	6·9	246

Distillation of Fractions A and B

No.	Wt., g.	Iodine value	Sapon. equiv.	No.	Wt., g.	Iodine value	Sapon. equiv.
A1	2·29	13·1	276·9	B1	2·68	112·4	280·7
A2	1·76	20·7	286·8	B2	2·58	150·1	291·6
A3	0·66*	—	361·2	B3	2·47	153·4	292·3
				B4	2·73	152·1	290·9
				B5†	2·76	150·1	292·9
				B6	2·66	146·5	292·3
				B7	1·35	141·5	293·9
				B8‡	1·91	102·6	298·3

* This fraction contained a further 0·066 g. of unsaponifiable material.

† B5 Acids; iodine value 158·3, $E^{1\%}_{1\,cm.}$ (180°/60 min.) at 234 mμ 717, at 268 mμ 1·9.

‡ 0·944 g. of this fraction contained 0·083 g. of unsaponifiable material.

Fraction C

Saponification equivalent of esters before acetylation 326·1; after acetylation 143·4; unsaponifiable material 1·40%: whence composition (% wt.) was calculated as dihydroxyoleic acid 90·8, non-hydroxy acids 7·8, unsaponifiable 1·4.

	Component acids					Excluding unsaponifiable		
	A	B	C	Unsaponifiable	Total	% (wt.)	% (mol.)	% (wt.)*
Palmitic	1·90	0·49	0·83	—	3·22	3·5	4·2	3·7
Stearic	0·73	0·51	0·43	—	1·67	1·8	2·0	1·9
Arachidic	0·48	—	0·17	—	0·65	0·7	0·7	0·7
Oleic	0·63	2·17	0·97	—	3·77	4·1	4·4	4·3
Linoleic	—	10·14	3·51	—	13·65	14·9	16·1	15·5
Dihydroxyoleic	—	0·07	68·83	—	68·90	75·0	72·6	—
Epoxyoleic	—	—	—	—	—	—	—	73·9
Unsaponifiable	0·06	0·12	1·06	6·90	8·14	—	—	—

* Since the quantity of epoxyoleic glyceride determined directly (74·1 mol.-%) exceeds the quantity of dihydroxyoleic acid (72·6 mol.-%), all the latter is considered to have been originally present as epoxyoleic glyceride. This final column gives the composition of the original acids on a weight basis.

(i) The saponification equivalent of the mixed esters was determined before (295·1) and after acetylation (263·2). These values, each determined in quadruplicate, indicate the presence of 15·2% of methyl hydroxyoctadecenoate in the mixed esters (15·3% calculated as acids). The absorption ($E^{1\%}_{1\,cm.}$ 266) at 234 mμ after isomerization (180°/60 min.) corresponds to 29·7% of linoleic acid after making allowance for unsaponifiable material. The balance (55·0%) consists of oleic and saturated acids, but the iodine value cannot be used to compute the content of oleic acid.

(ii) The mixed acids were partitioned by the procedure already described. The quantities of acids and of solvent were as before except that the acids were dissolved in the light petroleum and washed with seven successive portions of methanol (250 ml. each time). In this way 22·0 g. was extracted (5·13, 5·74, 4·20, 2·87, 1·91, 1·28, 0·92 g.), whilst 119·8 g. remained in petroleum

solutions 1–3 and 6·2 g. in petroleum solutions 4–5. The methanol extract (Fraction C, 14·9%) from the equivalent of its ester before (306·8) and after (179·8) acetylation contains 93·8% of hydroxyoctadecenoic acid. The remaining acids (fractions A and B, 85·1%) have iodine value 97·3, absorption ($E^{1\%}_{1\,cm.}$) at 234 mμ (180°/60 min.) 298, and still contains 2·65% of hydroxyoctadecenoic acid (this value was calculated from the value later found for fraction B alone). If it is assumed that this small quantity of hydroxy-acid does not appreciably affect the iodine value of this fraction, then its composition can be calculated in terms of hydroxyoctadecenoic, linoleic, oleic and saturated acids. The non-hydroxy-acids in fraction C are assigned the same composition as in fractions A and B. The results are given in Table II, column (ii).

(iii) A portion of fractions A and B was crystallized from methanol at — 20°. The insoluble acids (fraction A, 21·2% of the mixed acids) had iodine value 11·6 and the soluble acids (fraction B, 63·9%) had iodine value 126·7, $E^{1\%}_{1\,cm.}$ at 234 mμ (180°/60 min.) 384, and contained 3·53% of hydroxyoctadecenoic acid. Another set of results (Table II, column iii) is obtained by calculating fraction A as saturated and oleic acid on the basis of the iodine value ; fraction B as saturated, oleic, linoleic and hydroxyoctadecenoic acid ; and fraction C as hydroxyoctadecenoic and non-hydroxy-acids with the same composition as in fractions A and B together.

(iv) Finally fraction B was methylated, acetylated and distilled and the ester fractions examined as in the previous analysis of this oil.[3] (The fractionation data are not reproduced here.) The results for this fraction are combined with those for fraction A (obtained as in iii) and from fraction C (hydroxyoctadecenoic acid plus non-hydroxy-acids as in fractions A and B) to give the results given in Table II, column (iv).

Table II

Component acids of S. hispidus *seed oil (excluding unsaponifiable) as % of total acids*

	(i)*	(ii)*	(iii)*	(iv)*	(v)†
Saturated	55·0	21·4	19·8	21·2	21·0
Oleic		34·1	36·6	35·1	35·5
Linoleic	29·7	28·3	27·4	28·5	30·0
Hydroxyoctadecenoic	15·3	16·2	16·2	15·2	13·5

* For the significance of these see text
† Previous results[3]

In considering the distribution procedure as a method of examining oils containing monohydroxy-acids the results in column (iv) are relevant. There is a close accordance between the results obtained by this method and those of the previous analysis except that the figure for hydroxyoctadecenoic acid is now slightly higher at the expense of the linoleic acid content. The value previously reported may be low due to partial decomposition during distillation and, since decomposed acetoxyoctadecenoate would be calculated mainly as linoleate, the linoleic acid content would be correspondingly increased. We consider the present figures to be an improvement on those previously recorded and to show, further, that the method here described of separating hydroxy- from non-hydroxy-acids can be satisfactorily applied to oils containing relatively small amounts of monohydroxy-acids.

There are a number of ways in which the *Strophanthus* oils may be more quickly examined with varying degrees of accuracy. It is seen from Table II, column (i), that the method involving no separation of the acids gives good results which could be extended by determining the saturated acids independently by Bertram oxidation.[16, 17] Alternatively the hydroxy- and non-hydroxy-acids can be separated and examined independently. (It would be possible to take the methanol extract as wholly hydroxy-acid and at the same time neglect the small amount of this left in the petroleum extract ; the two errors so introduced would tend to cancel out.) This allows the oleic and saturated acids to be computed without resort to a Bertram oxidation but otherwise there seems to be little advantage unless a complete investigation is being made.

Glycol values

Much of the work just described would be facilitated by a quicker method of determining the content of hydroxy-acid and of distinguishing between various types of hydroxy-acids. For

these reasons we examined the methods of determining α-glycol based on the use of periodic acid.[18, 19] In our hands these methods were not entirely satisfactory, as high results were obtained with unsaturated compounds (cf. ref. 20), although there was considerable improvement when the reagent was a solution of potassium periodate in aqueous acetic acid used as described below, but even so the results are not more accurate than ± 1·5–2·0%. Some of our results are recorded in Table III from which it will be seen that the discrepancies observed are less marked with the triglycerides than with the acids or simple alkyl esters and that, as expected, the method cannot be used in the presence of epoxy-compounds.

Method.—The periodate solution is prepared by dissolving 1·4 g. of potassium periodate in water (200 ml.) and diluting to 1 l. with acetic acid; stronger solutions could not be prepared because of the low solubility of potassium periodate. The sample, the weight of which should be such that liberated iodine is not less than 80% of that liberated in the blank, is dissolved in 10 ml. of a mixture of acetic acid and chloroform (2 : 1) contained in a glass-stoppered vessel, with warming if necessary. To the solution at room temperature is added periodate solution (100 ml.) followed after thirty minutes by 10% potassium iodide solution (15 ml.) and distilled water (40 ml.). The liberated iodine is titrated with 0·1N-sodium thiosulphate solution. A blank is run at the same time omitting only the glycol.

$$\% \text{ glycol} = \frac{M(V_b - V_s)N}{20w}$$

where M = molecular weight of glycol, N = normality of thiosulphate, w = weight of sample, V_b and V_s are the amounts of thiosulphate required for the blank and the determination respectively.

Table III

Glycol values (as % dihydroxy-acid)

Reagent	KIO_4–H_2SO_4	HIO_4	KIO_4
Saturated acids	0·1–0·4		0·3–0·8
Methyl 12-hydroxystearate	1·5		1·5
Oleic acid	12–16	3·8	1·5–2·7
Methyl linoleate	19–28	3·9	1·9–3·8
Castor oil mixed acids	10		1·9
Groundnut oil	2·9		nil
Cottonseed oil	1·0		nil
Olive oil	0·8		nil
V. anthelmintica seed oil	77	17	7·4
Cephalocroton cordofanus seed oil	68	13	4·4
9 : 10-Dihydroxystearic acid (m.p. 95°)	103		101
9 : 10-Dihydroxystearic acid (m.p. 132°)	106		104
12 : 13-Dihydroxystearic acid (m.p. 95°)	102		97
12 : 13-Dihydroxyoleic acid (m.p. 54°)	162	111	102

Some derivatives of hydroxy-acids

In order to have some derivatives that might serve as useful reference compounds, the following compounds which have not previously been described were prepared.

9-*Hydroxyoctadec*-trans-12-*enoic acid.*—A concentrate of the naturally occurring *cis*-acid obtained from *Strophanthus* oils was isomerized by the method used by Kass & Radlove[21] for the isomerization of ricinoleic acid. The *trans* acid was obtained as small white needles from light petroleum (b.p. 40–60°), m.p. 57·5–59·5° (Found: C, 72·6; H, 11·1. $C_{18}H_{34}O_3$ requires C, 72·4; H, 11·5%).

9 : 12 : 13-*Trihydroxystearic acid.*—Oxidation of 9-hydroxyoctadec-*cis*-12-enoic acid with cold dilute alkaline permanganate gives two isomeric trihydroxystearic acids only one of which has been isolated in a pure state:[1] we have now purified the lower-melting isomer, which had m.p. 108–110° (Found: C, 64·9; H, 11·0. $C_{18}H_{36}O_5$ requires C, 65·0; H, 10·9%).

p-*Bromophenacyl esters.*—The *p*-bromophenacyl esters of the following acids were prepared by the standard procedure, the esters being crystallized from alcohol or aqueous alcohol.

9-Hydroxyoctadec-*cis*-12-enoic, m.p. 73·5–74·5° (Found: C, 63·1; H, 7·8; Br 16·2. $C_{26}H_{39}O_4Br$ requires C, 63·0; H, 7·9; Br, 16·1%); 9-hydroxystearic, m.p. 97·5–98° (Found: C, 62·6; H, 8·4; Br, 16·0. $C_{26}H_{41}O_4Br$ requires C, 62·8; H, 8·3; Br, 16·1%); and 12-hydroxystearic acid, m.p. 99·5–100° (Found: C, 62·8; H, 8·2; Br, 16·0%).

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319. *Fatty Acids. Part IV.* The Preparation of Eight 9 : 10 : 12 : 13-Tetrahydroxystearic Acids.†*

By K. E. Bharucha and F. D. Gunstone.

Naturally occurring epoxyoleic acid is proved to be an optically active *cis*-epoxide. It has been converted into *threo*- and *erythro*-12 : 13-dihydroxyoleic acid and, from these, eight optically active 9 : 10 : 12 : 13-tetrahydroxystearic acids have been prepared.

The relations between oleic and elaidic acid, the two racemic 9 : 10-dihydroxystearic acids derived from them, and various intermediates used in their preparation have now been made clear by Swern [1] who, using knowledge gained by several investigators, has been able to rationalise the stereochemistry of the processes involved. This work has opened the way to a similar explanation of the relation between linoleic acid (octadeca-*cis*-9 : *cis*-12-dienoic acid), its configurational isomers, and the tetrahydroxystearic acids derived from them. Although Swern's work removes certain theoretical difficulties concerning the relation between the tetrahydroxystearic acids and the dienoic acids, there remain several practical difficulties including that of obtaining pure linoleic acid, the fact that oxidation of linoleic acid or of its all-*trans*-isomer (linelaidic acid) always gives a mixture of two racemates separable only with difficulty and frequently giving eutectic mixtures, and the fact that direct oxidation of linoleic or linelaidic acids can give only four of the eight possible racemates so that indirect methods are required to obtain the other four racemates.

It is now accepted that linoleic acid present in seed oils is entirely the *cis-cis*-isomer,[2] that linelaidic acid is the *trans-trans*-isomer,[2] that oxidation by dilute alkaline permanganate involves *cis*-addition of hydroxyl groups to the double bond,[1] and that reaction with peracids or hydroxylation proceeding through the halogenohydrins is equivalent to *trans*-addition of hydroxyl groups.[1] The terms *cis* and *trans* used by several investigators to describe the resulting glycols are both confusing and incorrect when applied to open-chain compounds and should be replaced by *threo*- and *erythro*-. By definition *threo*-compounds result by *trans*-addition to a *cis*- or by *cis*-addition to a *trans*-ethylenic compound, whilst the *erythro*-isomers are the products of *cis*-addition to a *cis*- or *trans*-addition to a *trans*-ethylenic compound. 9 : 10 : 12 : 13-Tetrahydroxy stearic acid containing four asymmetric centres should exist in sixteen optically active forms and eight racemates. Eight stereoisomers are shown below (I—VIII), the other eight being represented by their enantiomorphs. These are obviously grouped in four pairs. It follows that *cis*-oxidation (dilute alkaline permanganate) of linoleic acid affords the two di-*erythro*-isomers which should also result from *trans*-oxidation of linelaidic acid; similarly the two di-*threo*-forms are produced from linoleic acid by *trans*-addition (performic acid) or from linelaidic acid by *cis*-addition, but the mixed *threo-erythro*-isomers (III—VI) cannot be produced directly from linoleic or linelaidic acids. In Table 1 the results obtained by several investigators are listed, the configuration of the products being determined on the basis of present views. The general agreement is immediately obvious.

Reference to the mixed *threo-erythro*-isomers is confined to reports by Kass and Burr [10] and by McKay and Bader.[13] The last authors claim to have prepared all eight racemic forms but owing to the known difficulties of working with these compounds we tried to prepare the eight racemates by an alternative procedure. We now report the preparation of eight tetrahydroxystearic acids which we consider to be optically active.

The starting materials for our oxidation experiments were the *threo*- and the *erythro*-form of 12 : 13-dihydroxyoleic acid which we obtained from 12 : 13-epoxyoleic acid (IX) known to occur in quantity among the component acids of *Vernonia anthelmintica* [15] (74%) and *Cephalocroton cordofanus* [16] (66%) seed oils. One isomer (X), m. p. 54°, resulted when

* Part III, *J.*, 1955, 3782.

† Geneva numbering, $CO_2H = 1$.

TABLE 1. *Di*-erythro- *and di*-threo-*tetrahydroxystearic acids.*

Date	Investigator	Reactant C_{18} acid	Reagent	M.p.s of di-*erythro*-acids		M.p.s of di-*threo*-acids	
1887	Hazura [3]	Linoleic *	$KMnO_4$	173°			
1909	Rollett [4]	,,	,,	171—173			
1912	Meyer and Beer [5]	,,	,,	173	162—163°		
1922	Nicolet and Cox [6]	,,	,,	170	153		
	,, ,,	,,	Hydrol. of halogeno-hydrin	—	—	144°	135°
1932	Smith and Chibnall [7]	,,	$KMnO_4$	172	159—160	—	—
1935	Green and Hilditch [8]	,,	,,	173	155	—	—
	,, ,,	,,	$AcOH–H_2O_2$	—	—	146	126
1937	Birosel [9]	,,	$KMnO_4$	174	164	—	—
1939	Kass and Burr [10]	Linelaidic	,,	—	—	146	122
1939	Riemenschneider *et al.* [11]	Linoleic	,,	174	163·5	—	—
1939	Hilditch and Jasperson [12]	,,	,,	173	157	—	—
	,, ,,	Linelaidic	,,	—	—	144	135
1948	McKay and Bader [13]	*erythro*-9 : 10-Dihydroxy-*cis*-12-en	,,	174	164	—	—
	,, ,,	*threo*-9 : 10-Diacetoxy-*cis*-12-en	$H·CO_2H–H_2O_2$	—	—	148	126
1954	McKay *et al.*[14]	Linoleic acid	,,	—	—	148	126
1956	Present work †	*erythro*-12 : 13-Dihydroxyoleic	$KMnO_4$	177	156	—	—
		threo-12 : 13-Diacetoxyoleic	$H·CO_2H–H_2O_2$	—	—	148	122

* The linoleic acid is either debrominated tetrabromostearic acid or a concentrate of oleic and linoleic acid prepared from a seed oil containing high proportions of the latter.

† The products obtained here are optically active (see text) whilst all the others are racemates.

TABLE 2. *Specific rotation.*

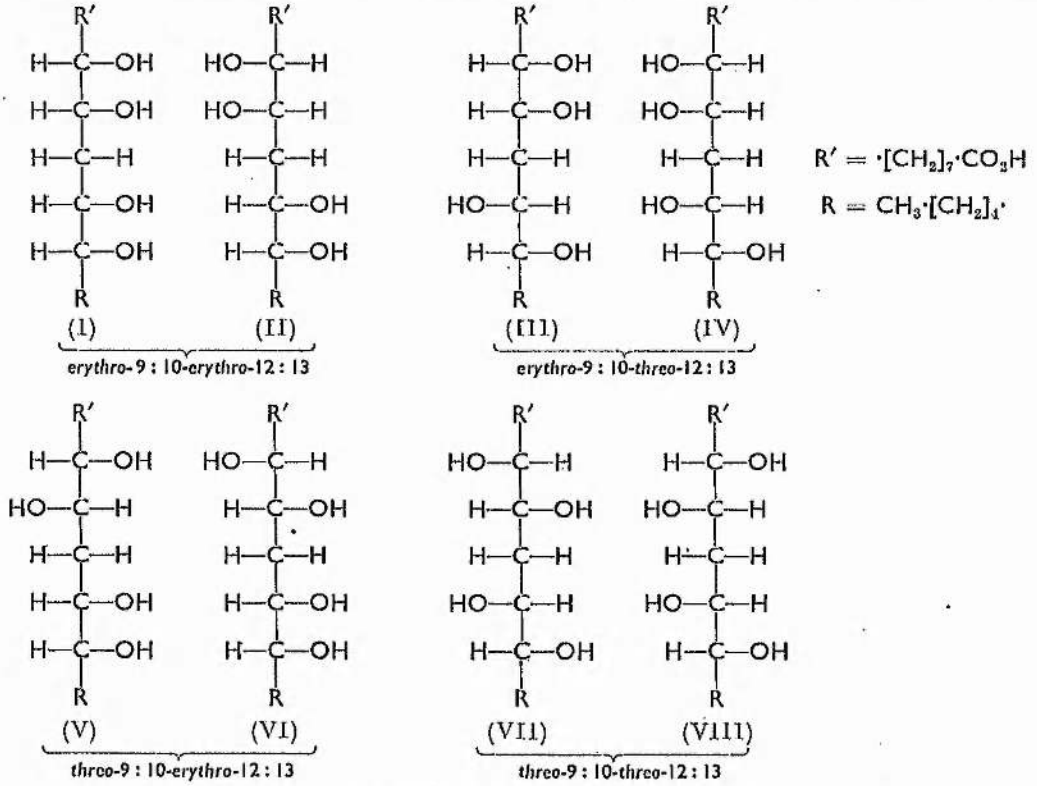
Acid	M. p.	Solvent: AcOH	Solvent: EtOH	M. p. of Me ester	Solvent: AcOH	Solvent: EtOH
threo-12 : 13-Dihydroxyoleic		**−6·8°** (19°, −0·69°)	**−5·7°** (19°, −0·51°)		—	—
erythro- ,, ...		**+1·2** (20, +0·12)	**−3·6** (20, −0·29)		—	—
threo-12 : 13-Dihydroxystearic.....		+1 (15·5, +0·01)	**−1·3** (20, −0·05)		—	—
erythro- ,, ...		+3 (16·5, +0·02)	**−1·7** (20·5, −0·03)		—	—
Tetrahydroxystearic acids :						
threo-9 : 10-*threo*-12 : 13-	148°	—	—		—	—
,, ,, ...	122	**−9** (17, −0·04)	0		0°	0°
erythro-9 : 10-*threo*-12 : 13-	165	−6 (20, −0·01)	—	136°	0	−1 (19, −0·01°)
,, ,, ...	112	—	—	—	—	—
threo-9 : 10-*erythro*-12 : 13-	156	**−44** (18·5, −0·35)	**−7** (20, −0·03)	145	**−46** (18·5°, −0·09°)	—
,, ,, ...	130	**+14** (17, +0·10)	**+5** (20, +0·03)	114	**+17** (20, +0·12)	**+22** (20, +0·08)
erythro-9 : 10-*erythro*-12 : 13-	177	**−10** (18·5, −0·04)	+6 (20, +0·01)	171	**−16** (18·5, −0·08)	**+22** (20, +0·05)
,, ,, ...	156	+14 (17, +0·02)	0	145	0	−2 (19, −0·01)
			Cephalocroton cordofanus seed oil		**+3·4** (16·5, +0·08)	—

Measurements were made with a 2 dm. tube and sodium light.

A dash (—) indicates that no measurement was made, 0 indicates a zero value.

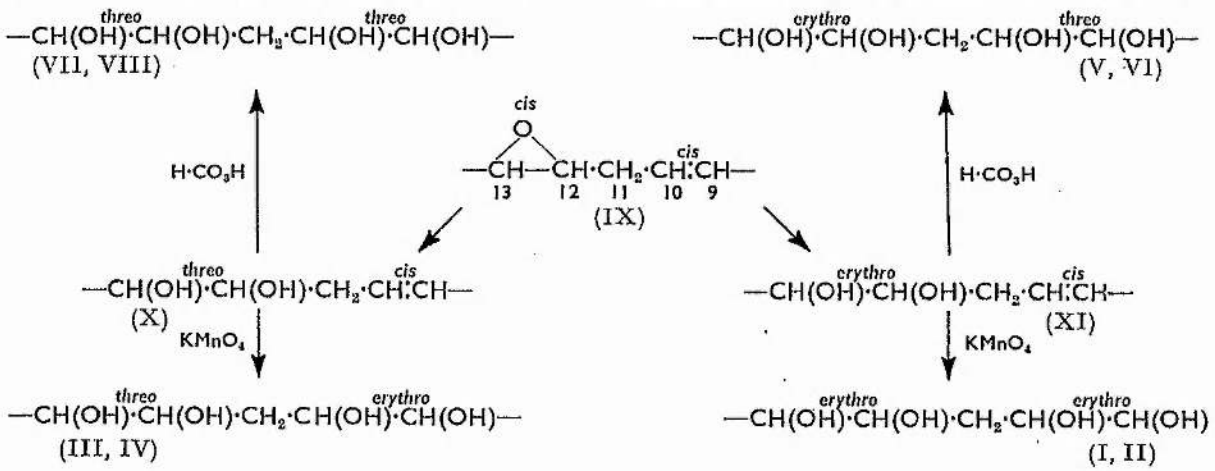
Values in heavy type are calculated from observed rotations of 0·03° or greater (some inactive dihydroxy- and tetrahydroxy-stearic acids gave observed rotations of only 0·00° or 0·01°); figures in parentheses are temperature and the observed rotation.

the epoxy-acid was treated with acetic acid and then with alkali. This has been proved to be a 12 : 13-dihydroxyoleic acid [15a] and was considered to be the *threo*-form since on hydrogenation it afforded a 12 : 13-dihydroxystearic acid (m. p. 95—96°) of similar m. p. (96·5—97°) to that of the known racemic *threo*-compound [17] and very different from that of the racemic *erythro*-isomer [17] (119—120°). We now find, however, that the unsaturated dihydroxy-acid is optically active (see Table 2) and, presumably, the saturated acid also,

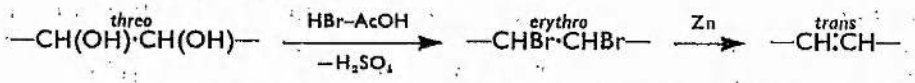


despite the low observed rotation; the identity of melting point between the racemic *threo*- and our dihydroxyoleic acid is thus inadequate proof of the *threo*-configuration of the latter and we now offer independent proof of this by the partial synthesis from it of octadec-*trans*-12-enoic acid.

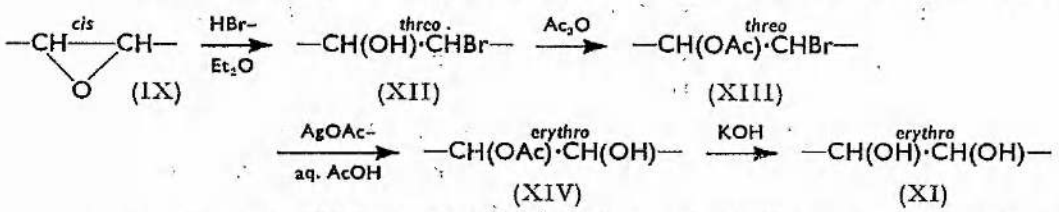
The dihydroxyoleic acid (X), when converted into the dibromide by Bowman's procedure [18] and subsequently debrominated, gave octadec-*trans*-12-enoic acid. This is quite certain since the melting point of this acid and of the two dihydroxystearic acids obtained from it agree with those expected of the *trans*-acid. Since the bromination occurs with inversion and debromination is a *trans*-elimination the dihydroxy-compound



must be the *threo*-form. This reaction also provides proof that the epoxide has the *cis*-configuration since ring opening is accompanied by inversion.[1]



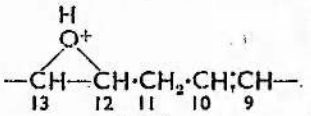
The preparation of *erythro*-12 : 13-dihydroxyoleic acid from the *cis*-epoxide requires a reaction sequence involving no inversion or an even number of inversions. This has been achieved by adaptation of some work of Winstein and Buckles [19] who, in investigations of neighbouring-group participation in replacement reactions, showed that interaction of silver acetate in dry acetic acid with several acetoxy-bromides proceeds with predominant retention of configuration, but that the presence of water in the solvent causes inversion to occur to an increasing extent, almost complete inversion taking place when one equivalent of water is present. We converted the *cis*-epoxide into the *threo*-bromohydrin (XII) which after acetylation gave the *erythro*-monoacetate (XIV) by reaction with



silver acetate in wet acetic acid, and this on hydrolysis afforded the required *erythro*-glycol (XI). The high overall yield of glycol resulting from this four-stage process (78% crude, 69% pure)—which also involves in the final stage removal of the other acids originally present in the oil—and the ease with which the glycol is obtained pure are evidence of the high stereospecificity of these reactions. We have simplified the procedure of Winstein and Buckles by reducing the reaction period with silver acetate from 8 to 2 hours and by effecting the reaction with silver nitrate in wet alcohol (cf. Bevan, Malkin, and Smith [20]).

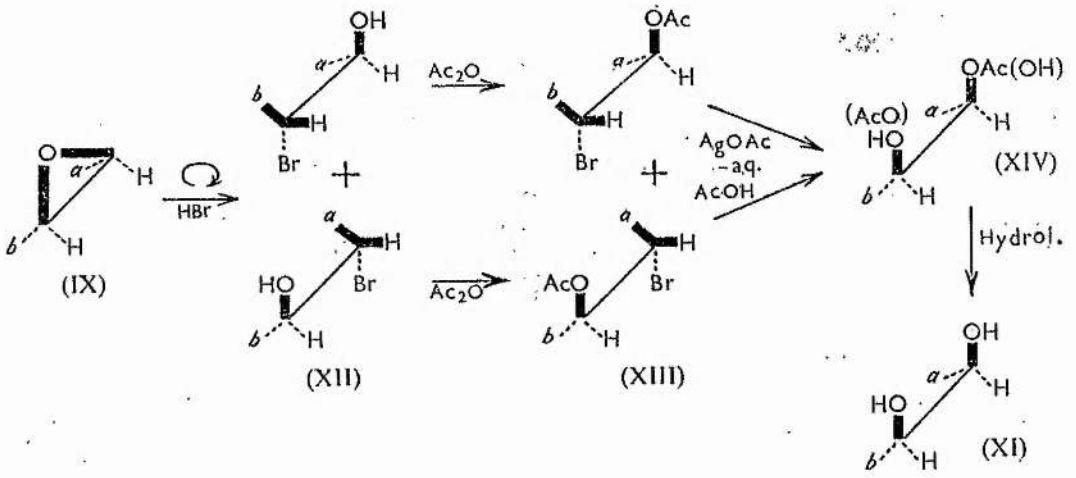
The *erythro*-dihydroxyoleic acid was a crystalline solid with m. p. (88°) greater than that of the *threo*-isomer (54°), like many similar compounds. Hydrogenation gave *erythro*-12 : 13-dihydroxystearic acid with a higher m. p. (126°) than that (119—120°) previously recorded for the racemic isomer.[17] This difference is due to the fact that our compound is optically active; the observed rotation is very small, but it is larger for its unsaturated precursor. The structures of the saturated and the unsaturated *erythro*-dihydroxy-acid were confirmed by oxidation.

Before considering how far the optical activity of these dihydroxy-acids is to be expected it is necessary to remark that optical activity of long-chain compounds is frequently so small that it cannot be measured and in the present instance this difficulty was sometimes increased by low solubilities (Baer and Fischer [21] showed that certain triglycerides which should be optically active lack observable rotatory power but are optically active when one or more of the usual aliphatic acyl groups is replaced by an aromatic acyl group). Epoxyoleic acid contains two asymmetric centres and the naturally occurring acid may be expected to be an enantiomorphic form and indeed must be if it is to give rise to optically active derivatives. We have now observed a small dextrorotation with *Cephalocroton cordofanus* seed oil. Consideration of the reaction mechanism involved in the preparation of the *erythro*-dihydroxyoleic acid suggests that an optically active epoxide should give an active glycol (see scheme). On the other hand, the active epoxide will give an active *threo*-glycol only if the conjugate acid (XV) is attacked unequally at $C_{(12)}$ and $C_{(13)}$ by the acetate ion: an attack equally distributed between these two centres would give a racemic glycol. Although the specific rotation of these two acids is fairly small the observed rotations are definite (see Table 2). The values for the corresponding saturated acids are less definite, but as hydrogenation does not affect the active centres racemisation would not be expected to occur; further the m. p. of the saturated *erythro*-isomer differs appreciably from that of the racemic compound.



(XV)

The two dihydroxyoleic acids have been oxidised with dilute alkaline potassium permanganate and with performic acid. In each case the product is a mixture which has been separated by an extensive series of crystallisations. Some general comments are made before discussing the results.



$a = CH_3\cdot[CH_2]_4\cdot$ $b = \cdot CH_2CH{:}CH\cdot[CH_2]_7\cdot CO_2H$

(i) In the performic acid oxidation of *threo*-dihydroxyoleic acid the main product was a low-melting, ether-soluble compound which accompanied the desired tetrahydroxy-acids. Two individual compounds were isolated from this product (m. p.s 77·5—78°, 94—95°) but these were not identified. Similar products have been isolated by other investigators,[8,14] whilst Paul and Tchelitcheff[22] have reported that furan compounds are formed in the hydrolysis of 1 : 2 : 4 : 5-diepoxypentane. We found little or none of these products when we oxidised diacetoxyoleic acid and this became our standard procedure.

(ii) The separation of the two products obtained in each reaction involves many crystallisations. The higher-melting isomer being the less soluble is generally the more easily isolated; purification of the lower-melting isomer was more difficult and we are less confident of the homogeneity of the latter though in all cases the products were crystallised to constant melting point.

(iii) McKay and his colleagues[14] report that the two tetrahydroxy-acids are more easily separated as their methyl esters and in some cases we have checked our results by separation of both acids and esters with subsequent hydrolysis of the separated esters.

(iv) We emphasise the purity of our starting materials. Both 12 : 13-dihydroxyoleic acids are crystalline solids, readily purified and available in quantity from the sources already mentioned. This is in contrast to much of the previous work on this problem which has been undertaken with impure starting materials. Many investigators have used a concentrate of linoleic acid obtained from a suitable source and containing appreciable quantities of oleic acid. Alternatively, linoleic acid has been prepared by debromination of tetrabromostearic acid and is known then to contain small quantities of conjugated isomers and larger quantities of goemetrical isomers of linoleic acid. In either case the desired product is contaminated with closely related compounds. McKay and Bader[13] used *threo*- and *erythro*-9 : 10-dihydroxyoctadec-12-enoic acid, prepared from linoleic acid by partial bromination at the 12 : 13-positions, oxidation of the 9 : 10-double bond by permanganate or per-acid, and subsequent debromination. We consider these acids, isomeric with our dihydroxyoleic acids, should be solid, though McKay and Bader obtained only the *erythro*-isomer as a solid. Although their oily *threo*-compound was hydrogenated to *threo*-9 : 10-dihydroxystearic acid we consider it unlikely that it was pure.

Details of the oxidations are given in the Experimental section and the results are summarised in Tables 2 and 3. The high yield from the performic acid oxidation is in accordance with that usually obtained with monoethenoid compounds[23] and is greater

TABLE 3. 9 : 10 : 12 : 13-*Tetrahydroxystearic acids.*

	threo-9 : 10-*threo*-12 : 13- (VII, VIII)		*erythro*-9 : 10-*threo*-12 : 13- (III, IV)		*threo*-9 : 10-*erythro*-12 : 13- (V, VI)		*erythro*-9 : 10-*erythro*-12 : 13- (I, II)	
Yield of mixed tetrahydroxystearic acids (%)	82		61		92		59	
Yield of separated acids :								
(i) separated as acids (%) *	8	34	17	6	21	41	24	13
(ii) separated as esters (%) *	—	—	20	1·1	25	25	26	17
Acid, m. p.	147·5—148·5°	121—122°	164—165°	**112—113°**	**156·5—157°**	**129·5—131°**	176·5—177°	**155·5—156·5°**
C (%) †	62·1	62·0	62·0	62·2	62·1	61·9	62·2	62·1
H (%) †	10·5	10·3	10·6	10·1	10·6	10·6	10·4	10·5
Methyl ester, m. p.	—	—	135·5—136°	—	144·5—145·2°	113·5—115°	**170·5—171°**	143—146°
C (%) ‡	—	—	62·8	—	62·8	63·0	62·8	63·1
H (%) ‡	—	—	10·3	—	10·6	10·8	10·7	10·3
Racemic compound : [13, 14]								
acid, m. p.	148°	126°	164°	126°	164°	142°	174°	164°
ester, m. p.	118°	95°	—	—	—	—	157°	145·5°

* Based on the total theoretical yield. † $C_{18}H_{36}O_6$ requires C, 62·0, H, 10·4%.
‡ $C_{19}H_{38}O_6$ requires C, 63·0; H, 10·6%.
M. p.s in heavy type differ appreciably from those quoted for the racemic compound.

than in the oxidation of linoleic acid.[14, 24] The yields of separated tetrahydroxy-acids are much higher than those reported by McKay and Bader.[13]

We consider our products to be optically active for three reasons. The starting materials are active and it is believed that oxidation will not affect the asymmetric centres present; thus, the 9 : 10 : 12-trihydroxystearic acids obtained by oxidation of ricinoleic acid are optically active.[25] Several, though not all, of our tetrahydroxystearic acids show significant optical rotation (this refers to the observed rotation rather than to the calculated values of specific rotation). In some cases there is a marked difference in m. p. between the active and the inactive forms (see Table 3). The last may not be very strong evidence in all the instances cited since we consider some of the values quoted by McKay and Bader to be unsatisfactory, but one case is particularly strong, *viz.*, the high-melting form of methyl *erythro*-9 : 10-*erythro*-12 : 13-tetrahydroxystearate. We have confirmed McKay's value of 157° for the racemate whilst our active form has m. p. 171°.

In view of the frequency with which the m. p.s of active and inactive compounds of this type resemble one another it is interesting to relate our results to some conclusions by Kass and Burr [10] which have been questioned by McKay and Bader.[13] Kass and Burr obtained, from elaidinised linoleic acid, linelaidic acid and a liquid isomer considered to be the *trans*-9 : *cis*-12-acid from which they prepared two tetrahydroxystearic acids (m. p. 156—158°, 126—127°) by oxidation with dilute alkaline permanganate, presumably the *threo*-9 : 10-*erythro*-12 : 13-acids. McKay and Bader, however, report 164° and 142° for these acids (these were prepared from the *liquid* dihydroxyoctadecenoic acid and the higher-melting acid did not depress the m. p. of the higher-melting form of the *erythro*-9 : 10-*threo*-12 : 13-acid though the two are reported to have different crystalline properties) and consider that Kass and Burr's products are the *erythro*-9 : 10-*threo*-12 : 13-acids (m. p. 164°, 126°) which would be derived from the *cis*-9 : *trans*-12-octadecadienoic acid. On the other hand, our results (156°, 130°) suggest that Kass and Burr's original view may be correct, but since our products are enantiomorphic and theirs racemic this is not certain.

EXPERIMENTAL

threo-12 : 13-*Dihydroxyoleic Acid* (X).—This was isolated from *Vernonia anthelmintica* or *Cephalocroton cordofanus* seed oil by treatment with (i) acetic acid and (ii) alcoholic alkali as previously described.[15a] The dihydroxy-acid (m. p. 52—55°; 57% based on epoxy-acid present) was isolated by crystallisation from ether–light petroleum (b. p. 40—60°; 1 : 1) and then from ethyl acetate.

Partial Synthesis of Octadec-trans-12-*enoic Acid.*—*threo*-12 : 13-Dihydroxystearic acid [15a] (5 g.) was kept at room temperature for 16 hr. with a solution of hydrogen bromide in acetic

acid (50 ml.; *d* 1·25) and concentrated sulphuric acid (5 ml.) and then heated to 100° for 8 hr., further hydrogen bromide reagent (5 ml.) being added after 4 hr. The solution was then cooled, diluted with water, and extracted with light petroleum (b. p. 60—80°). The crude *dibromide* (6·73 g., 97%) was purified by crystallisation from light petroleum (b. p. 40—60°) (5·13 g., 74%), m. p. 47·5—48·5° (Found: C, 49·0; H, 7·4; Br, 36·4. $C_{18}H_{34}O_2Br_2$ requires C, 48·9; H, 7·75; Br, 36·1%).

The dibromostearic acid (4·0 g.) was added to a mixture of zinc dust (8·7 g.), methanol (45 ml.), and aqueous hydrogen bromide (50%; 0·8 ml.) which had been refluxed for 5 min. and the whole boiled for 1 hr. in a nitrogen atmosphere. The filtered solution was extracted with ether; the extract, after being washed with alkali, contained only 0·07 g. of material, showing that practically no esterification had occurred. The alkaline solution when acidified and extracted gave the crude *trans*-acid (1·66 g., 65%) which after several crystallisations from methanol and acetone had m. p. 52—53° (lit.,[17] 52—53°) (Found: C, 76·6; H, 12·1. Calc. for $C_{18}H_{34}O_2$: C, 76·5; H, 12·1%).

This was converted into *threo*-12 : 13-dihydroxystearic acid, m. p. 96—97° (lit.,[17] 96·5—97°), by dilute alkaline permanganate and into the *erythro*-isomer, m. p. 117—118° (lit.,[17] 119—120°), by performic acid (Found: C, 68·1; H, 11·5. Calc. for $C_{18}H_{36}O_4$: C, 68·3; H, 11·5%).

erythro-12 : 13-*Dihydroxyoleic Acid* (XI).—*Cephalocroton cordofanus* seed oil (53 g.) was kept overnight in ether (1·5 l.) saturated with hydrogen bromide, and the solution was then washed with water and dried, and the solvent removed. The resulting bromohydrin (62 g.) was acetylated by boiling acetic anhydride (300 ml.) for 3 hr. and then heated with water (200 ml.) for a further 30 min., the product (64 g.) being isolated by ether-extraction. Silver acetate [prepared by slow addition of a solution of potassium acetate (20·8 g. in 75 ml. of water) to one of silver nitrate (32·8 g. in 75 ml. of water) and by subsequent washing of the precipitate with cold water and then with acetic acid] was added to the oily acetoxy-bromide (59 g.) dissolved in acetic acid (325 ml.) containing a little water (3·3 ml.) and refluxed for 8 hr. After filtering, the cold reaction mixture was diluted with water and extracted with ether, and the solvent removed from the extract after it had been washed and dried. The residue (54 g.) was finally hydrolysed with N-alcoholic potassium hydroxide (1·5 l.), and the mixed acids (45 g.) were recovered by ether-extraction and then crystallised from ether. The erythro-12 : 13-*dihydroxyoleic acid* (21·3 g., 69% overall yield based on epoxy-acid originally present) was practically pure after the first crystallisation (m. p. 87—88°) (Found: C, 68·5; H, 10·8. $C_{18}H_{34}O_4$ requires C, 68·75; H, 10·9%).

The reaction proceeded equally smoothly with *V. anthelmintica* seed oil and we found (i) that the period of heating with silver acetate could be reduced to 2 hr. with an increase in yield (82%) and (ii) that a similar reaction occurred (45% in a single experiment) when the acetoxy-bromide was refluxed for 1 hr. with its own weight of silver nitrate in aqueous alcohol (10 ml. per g. of acetoxy-bromide).

The following derivatives were prepared by standard procedures: *methyl ester*, m. p. 56—57·5° (Found: C, 69·2; H, 11·2. $C_{19}H_{36}O_4$ requires C, 69·5; H, 11·1%); *ethyl ester*, m. p. 52—53° (Found: C, 70·1; H, 11·3. $C_{20}H_{38}O_4$ requires C, 70·1; H, 11·2%); p-*bromophenacyl ester*, m. p. 116—117° (Found: C, 61·1; H, 7·6; Br, 15·8. $C_{26}H_{39}O_5Br$ requires C, 61·1; H, 7·7; Br, 15·6%).

When hydrogenated over 5% palladium–charcoal the *erythro*-dihydroxyoleic acid (100 mg.) gave erythro-12 : 13-*dihydroxystearic acid* (90 mg.), m. p. 125—126° raised only to 125·5—126·5° after three crystallisations from ethanol and from ethyl acetate (Found: C, 68·3; H, 11·3. $C_{18}H_{36}O_4$ requires C, 68·3; H, 11·5%). The following esters were prepared: *methyl*, m. p. 102—103° (Found: C, 69·2; H, 11·6. $C_{19}H_{38}O_4$ requires C, 69·1; H, 11·6%); *ethyl*, m. p. 97—98° (Found: C, 69·7; H, 11·5. $C_{20}H_{40}O_4$ requires C, 69·7; H, 11·7%); p-*bromophenacyl*, m. p. 136·5—137·5° (Found: C, 60·6; H, 8·1; Br, 15·5. $C_{26}H_{41}O_5Br$ requires C, 60·8; H, 8·1; Br, 15·6%).

Oxidised[15a] with potassium permanganate in acetic acid solution at 45—50° *erythro*-12 : 13-dihydroxyoleic acid (1·80 g.) gave hexanoic acid (0·59 g.) (*p*-bromophenacyl ester, m. p. and mixed m. p. 70·5—71·5°; 63—64° when mixed with the ester of heptanoic acid), and azelaic acid (0·59 g.), m. p. and mixed m. p. 105—106°.

erythro-12 : 13-Dihydroxystearic acid (1·01 g.), similarly treated, afforded hexanoic acid (0·16 g.) (*p*-bromophenacyl ester, m. p. and mixed m. p. 71·5—72°; 62—63° when mixed with the ester of heptanoic acid), and dodecanedioic acid (0·23 g.), m. p. and mixed m. p. 129—131° (112—114° when mixed with sebacic acid).

erythro-9 : 10-threo-12 : 13-*Tetrahydroxystearic Acids* (III, IV).—*threo*-12 : 13-Dihydroxy-

oleic acid (5 g.), oxidised with dilute alkaline permanganate (potassium permanganate, 5·5 g.; sodium hydroxide, 5·0 g.) according to the procedure of Lapworth and Mottram,[26] gave crude tetrahydroxystearic acids (3·36 g.), insoluble in light petroleum (b. p. 40—60°).

A portion of these (1·60 g.) was separated by utilising the lower solubility of the higher-melting *acid* in ethyl acetate. This (437 mg.), after crystallisation from ethanol, melted at 164—165° (*methyl ester*, m. p. 135·5—136°). The lower-melting isomer, after repeated crystallisation from ethyl acetate and finally acetone, melted at 111—113°.

The remainder of the mixed acids (1·7 g.) was methylated (methanolic hydrogen chloride) and the methanol solution on progressive concentration gave several fractions of the higher-melting ester (576 mg.), m. p. 135—135·5°. The mother-liquors gave a small amount of solid, hydrolysed to the *acid* melting at 112—113°.

threo-9 : 10-threo-12 : 13-*Tetrahydroxystearic Acids* (VII, VIII).—*threo*-12 : 13-Dihydroxyoleic acid (6 g.) was boiled for 4 hr. with acetic anhydride (50 ml.) and for a further 2 hr. after the addition of water (50 ml.), and the product (7·23 g.) was isolated by ether-extraction. (The original dihydroxyoleic acid could be recovered in good yield after alkaline hydrolysis.) The diacetoxyoleic acid (5·4 g.), oxidised with formic acid (98%; 13 ml.) and hydrogen peroxide (25%; 2 ml.) by Swern's procedure,[23] gave a mixture of the di-*threo*-acids (4·54 g.) of which the greater part (3·87 g.) was insoluble in ether. This was extracted with acetone (6 × 50 ml.), and the last two extracts deposited, at room temperature, material (402 mg.) of m. p. 144—146° which was raised to 147·5—148·5° by several crystallisations from acetone and from ethyl acetate. The first two extracts gave crystals (2·12 g.) of m. p. 119—123° raised to 121—122° (1·59) by repeated crystallisation from acetone and from ethyl acetate.

When the dihydroxyoleic acid (5 g.) was similarly oxidised without prior acetylation the crude product (4·6 g.) gave a large ether-soluble fraction (2·53 g.; m. p. 63—68°) from which two products were isolated by fractional crystallisation : (*a*) m. p. 77·5—78·5° (Found : C, 65·2; H, 10·1. Calc. for $C_{18}H_{34}O_5$: C, 65·4; H, 10·4%); and (*b*) m. p. 94—95° (Found : C, 65·1; H, 10·0%). Our product (*b*) depressed the m. p. of compound A [14] (m. p. 95·5—96°) to 82—84° and of compound B [14] (m. p. 90—91°) to 81—83°.

erythro-9 : 10-erythro-12 : 13-*Tetrahydroxystearic Acids* (I, II).—*erythro*-12 : 13-Dihydroxyoleic acid (3 g.), dissolved in water (300 ml.) containing sodium hydroxide (3 g.), was diluted with 2·4 l. of water and then oxidised at 10° with potassium permanganate solution (1%; 290 ml.). After 5 min. the solution was decolorised with sulphur dioxide and, when acidified, gave crude tetrahydroxystearic acids (2·04 g.) of which the greater part (1·97 g.) was ether-insoluble. This was dissolved in ethanol and that part which crystallised at room temperature (1·39 g.), after several extractions with ethyl acetate and with acetone in which the *acid* is insoluble, was finally crystallised from aqueous ethanol, m. p. 176·5—177° (780 mg.); the *methyl ester* had m. p. and mixed m. p. with sample described below 170·5—171°. The low-melting *acid* was obtained from the ethanol mother-liquors and from the ethyl acetate and acetone extracts; a long series of crystallisations from ethyl acetate, aqueous alcohol, water, chloroform–acetic acid, acetone, and ethanol did not raise the m. p. above 155·5—156·5° (440 mg.). This was depressed to 145—150° on admixture with a sample of the *threo*-9 : 10-*erythro*-12 : 13-acid (m. p. 157°).

A sample of the mixed tetrahydroxy-acids (3·6 g.) was methylated by boiling it with methanolic hydrogen chloride. The product in methanol (150 ml.) deposited a large fraction at room temperature (2·14 g.; m. p. 159—165°) and a further fraction at 0° (0·96 g.; m. p. 143—144°). The m. p. rose sharply to 169—171° (1·50 g.) when the former was recrystallised from the same solvent and remained unchanged when the ester was washed with alkali; hydrolysis afforded the di-*erythro*-acid, m. p. 176·5—177·5°. The low-melting *ester* was unchanged in m. p. after several crystallisations or after washing with alkali; hydrolysis gave the low-melting di-*erythro*-acid.

The two racemic di-*erythro*-acids (m. p. 171·5—172·5°, 154—156°) were prepared by oxidation of linoleic acid; the methyl ester of the higher-melting acid had m. p. 157—157·5° confirming the value given by McKay *et al.*[14]

threo-9 : 10-erythro-12 : 13-*Tetrahydroxystearic Acid* (V, VI).—*erythro*-12 : 13-Dihydroxyoleic acid (5 g.) was acetylated and the product (6·23 g.) oxidised with performic acid, as already described. The greater part (5·02 g.) of the product (5·13 g.) was insoluble in ether.

A portion of the acids (2·5 g.), extracted with acetone and then with ethyl acetate, gave the high-melting *acid* as an insoluble fraction (574 mg.), m. p. 155—158° which became 156·5—157° after crystallisation from ethanol and repeated extraction with acetone and with ethyl acetate. The crystals which separated from the first acetone extracts were combined (1·27 g.; m. p.

128—131°). After repeated crystallisation from ethyl acetate and from ethanol this *acid* had m. p. 129·5—131°.

The remaining acids were esterified with cold methanolic hydrogen chloride, and the solution, when concentrated, gave crystals of *ester* (792 mg.), m. p. 142—145° raised to 144·5—145·5° after crystallisation from acetone, ethyl acetate, and methanol. Hydrolysis gave the higher-melting acid, m. p. and mixed m. p. 155·5—157°. Further concentration of the original methanol solution gave crude samples (1·63 g.) of the low-melting *ester*, purified by crystallisation from acetone, ethyl acetate, and methanol to m. p. 113·5—115° (720 mg.). Hydrolysis gave the low-melting acid, m. p. and mixed m. p. 130—131°.

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